



**THE UNIVERSITY OF READING**

**PROCESSING OF CASSAVA, RESIDUAL CYANOGENS AND  
MYCOTOXIN CONTENT IN TRADITIONALLY PROCESSED  
CASSAVA PRODUCTS**

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## ABSTRACT.

Cassava samples from some villages in Tanzania processed by wet fermentation, solid-state fermentation and sun drying were analysed for residual cyanogens and presence of mycotoxins. Cassava samples (bitter varieties) processed by wet and solid-state fermentation had residual cyanogens of 5.84 mg HCN.kg<sup>-1</sup> and 13.95 mg HCN.kg<sup>-1</sup> respectively. Sun dried cassava samples (sweet varieties) had 6.8 mg HCN.kg<sup>-1</sup>. It was concluded that wet fermentation is very effective in reducing cyanogens in cassava. No mycotoxins (aflatoxins) were detected in cassava samples.

The chemical composition of these cassava samples was also determined. Wet fermentation showed lower content of vitamin C, reducing sugars and protein compared to samples processed by solid state fermentation and sun drying.

In another experiment, cassava flakes were produced on a drum drier using varying pre-cooking temperatures and drum speeds. Pre-cooking conditions were: no pre-cooking, pre-cooking at 75°C for 35 minutes and 100°C for 5 minutes. The drum speeds used were 11.5 and 14.0 r.p.m. which correspond to 4.0 and 3.4 seconds drying time respectively. The flakes were analysed for vitamin C, moisture, free starch, reducing sugars and protein content.

Pre-cooking conditions affected vitamin C, moisture and free starch content while drum speed affected only the moisture content of the flakes. Prolonged pre-cooking time caused losses in vitamin C while pre-cooking at the higher temperature increased free starch content of the flakes. The moisture content increased with increasing drum speed.

The soluble amylose test showed that starch retrogradation occurs when cassava is cooled after the pre-cooking stage.

The textural characteristics of reconstituted mash from the flakes was analysed using the Texture Analyser, after adding emulsifiers (stearate monoglyceride, sodium caseinate and skim milk powder). Results from Texture Profile Analysis (TPA) (adhesiveness), Back extrusion and sensory evaluation tests showed that stearate monoglyceride emulsifier reduces the stickiness of reconstituted cassava flakes mash. The Differential Scanning Calorimetry (DSC) thermograms indicated the formation of an amylose-monoglyceride complex. Cassava samples with stearate monoglyceride had low water absorption capacity and high bulk density. Also sample with stearate monoglyceride had low viscosity according to the results from the Brabender amylograph tests.

Samples of cassava flakes and reconstituted mash with skim milk powder and sodium caseinate were darker in colour compared to those with stearate monoglyceride according to the results from both instrumental (Hunter Lab spectrophotometer) and sensory tests.

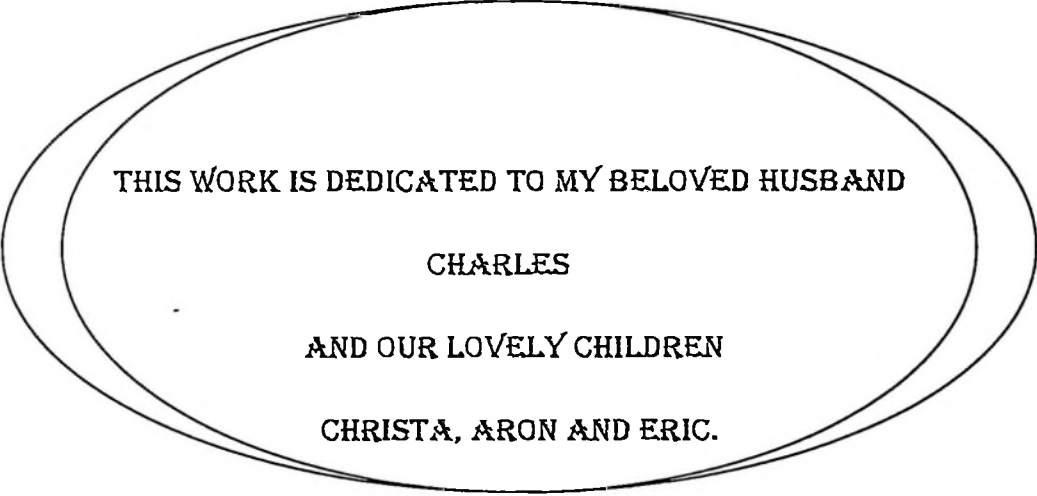
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THIS WORK IS DEDICATED TO MY BELOVED HUSBAND

CHARLES

AND OUR LOVELY CHILDREN

CHRISTA, ARON AND ERIC.

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## Chapter 1

### INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a starchy root crop that is grown almost entirely within the tropics. Although it is one of the most important crops in the tropical countries, it is little known elsewhere, and within the tropics, it is considered to be a low grade subsistence crop (Cock, 1985).

Cassava is known by many different names. In Brazil it is known as mandioca or aipim, in the French-speaking world as manioc, in Spanish America as yuca, in Malaysia, Sri Lanka and India as tapioca and in the rest of English-speaking world as cassava (Cock, 1985).

Cassava is grown chiefly as human food, but it is also an important animal feed, and it has several industrial uses. As one of the cheapest sources of food energy, cassava should play a major role in meeting developing countries' rising consumption of both food and animal feed (Nartey, 1978).

Raw cassava contains the cyanogenic glucosides, linamarin and lotaustralin, which are converted to hydrogen cyanide (HCN), a poison, when they come in contact with linamarase, an enzyme that is released when cells of the cassava roots are ruptured. Lancaster *et al* (1982) said that although occasionally deaths from consuming raw cassava have been reported, the traditional processing and cooking methods reduce the cyanogen levels. If normal preparation procedures are used, acute cyanide toxicity does not occur.

Chronic cyanide toxicity occurs in some localities in Africa, where cassava consumption

is high, up to the equivalent of 1 kilogram or more of fresh cassava roots per day over a long period, and where, the consumption of iodine and protein is extremely low (Nartey, 1978).

In order to minimise the cyanogens content, cassava is processed by different traditional methods, which include fermentation (wet and solid-state) and sun drying. However, in solid-state fermentation and sun drying, there is proliferation of spoilage and toxigenic micro-organisms on cassava, some of which may produce mycotoxins.

Fermented foods are an important part of the diet of people in many parts of the world. Unfortunately, where mould contamination of food occurs, some mycotoxins may be produced. Some of the mycotoxins produced are, aflatoxins, ochratoxins, patulin and zearalenone (Soares and Rodriguez-Amaya, 1989).

There are a number of reports of aflatoxins in African cassava products. The other mycotoxins which have been reported to be found in cassava products are zearalenone and ochratoxin A (Soares and Rodriguez-Amaya, 1989).

In Tanzania, most of the non-grain starch staples e.g. cassava, have received minimum attention in research, because of lack of an organised marketing system. they are mainly used as subsistence crops. A lot of attention has been paid to cereals like maize, rice etc., which are used as food and cash crops as well. The current food problems are revealing the importance of such crops, e.g. cassava. The increase in population, especially in urban areas, causes the increased demand for such crops, which were originally not preferred by many people. Since these crops may enter the inter-regional trade market, due to the

growing demand for food in urban areas, there is a need for research to be carried out on them.

The main objective of this study is to investigate the presence and extent of residual cyanogens and some mycotoxins in traditionally processed cassava products in Tanzanian villages.

Another objective is to develop other product types from cassava by using a dehydration process, e.g. (compared to dehydrated potato products). The work involves preparation of cassava flakes.

The nutritional composition of cassava flakes was determined to see whether they are affected by the processing conditions.

The effects of processing were determined on:

Vitamin C content.

Free starch content.

Reducing sugars content.

Colour, (degree of non-enzymatic browning).

Protein content.

Moisture content.

The rehydration properties, textural and sensory characteristics of the flakes were also investigated.

## Chapter 2

### LITERATURE REVIEW

#### *Cassava (Manihot esculenta)*

##### Classification

Cassava, *M. esculenta* belongs to the family Euphorbeaceae. This family contains about 300 genera, about 5,000 species, most of which are tropical, including two very important species, the castor plant (*Ricinus communis*) and the para rubber (*Hevea brasiliensis*) (Doku, 1969).

Different botanists, have classified cassava in different ways. The two species recognised were:

1. A poisonous, bitter species, *Manihot esculenta* Crantz and,
2. A sweet non-poisonous species *M. duleis* Baill.

This classification is based on the cyanogenic glucosides content of roots, considering the sweet species to have lesser cyanogenic glucosides than the bitter species. (Doku, 1969).

However, Bolhius (1954) suggested that such a classification cannot be very useful, as the environment also has an effect on root toxicity. Drought and low soil fertility status as well as a deficiency of potassium are known to increase the cyanogenic glucosides content, hence the bitterness and toxicity of roots. So in that case, there is a tendency to regard all cassava as varieties of *M. esculenta* Crantz.

The cassava plant is a perennial shrub. The height ranges from about 1-3.5 m, depending on the variety and growing conditions. The mature stems and branches are rounded and generally of uniform thickness, not more than 5-7 cm diameter.

Cuttings are main propagation method, but seedlings can also be used although they can take up to 2-3 years to produce mature roots (Doku, 1969).

### **Origin and Dissemination**

Cassava is a native of South America, its origin probably being Brazil where it had been an important source of food. It was introduced from Brazil to the tropical areas of Africa, the Far East and the Caribbean Islands by the early Portuguese slave dealers in the 16th and 17th centuries (Jones, 1959).

### **Chemical and Nutritional Composition**

Cassava is an excellent source of dietary energy (565 Kj). The fresh roots have 30-40g/100g dry matter. Protein content is very low, about 1.2 g/100g dry solids with fatcontent even lower, between 0.2-0.5 g/100g dry solids (Cock, 1985; Grace, 1977).

The Vitamin C content ranges between 34-36 mg/100g dry solids. It contains significant amounts of thiamine (vitamin B<sub>1</sub>) riboflavin (vitamin B<sub>2</sub>) and niacin. Cassava roots have higher dietary energy, total carbohydrate and vitamin C content compared to other root crops as shown in

Table 2-1.

Table 2-1 The nutritional composition of tropical root crops  
(per 100g of edible portion)

Crop	Food energy Kj	Moisture (%)	Protein (g)	Fat (g)	Protein/ energy <sub>a</sub> ratio (g/1 000 Kcal)	Fibre (g)	Total CHO & fibre (g)	Ash (g)	Ca (mg)	P (mg)	Fe (mg)	K (mg)	Na (mg)	Caro- tene equi. (µg)	Thia- min (mg)	Ribo- flavin (mg)	Nia- cin (mg)	Ascor- bic acid (mg)	Folic acid (µ)
Cassava	565	65.5	1.0	0.2	7	1.0	32.4	0.9	26	32	0.9	394	2	0	0.05	0.04	0.6	34	24.2
Sweet potatoes																			
white	452	72.3	1.0	0.3	15	0.8	25.1	0.7	21	50	0.9	210	31	35	0.14	0.05	0.7	21	52.0
yellow	481	70.0	1.2	0.3	-	0.8	27.1	0.7	36	56	0.9	304	36	1 680	0.12	0.05	0.6	30	-
Potato	335	78.3	2.0	0.1	27	0.4	19.1	0.9	9	55	0.7	451	7	30	0.11	0.04	1.2	14	-
Yam	452	71.8	2.0	0.1	21	0.5	25.1	1.0	22	39	1.0	294	10	0	0.10	0.04	0.07	-	-
Taro and tamlia	393	75.4	2.2	0.4	-	0.8	21.0	1.0	34	62	1.2	448	10	tr.	0.12	0.04	1.0	8	-
Glant taro	255	83.0	0.6	-	-	-	14.8	-	30	50	1.0	-	-	0	0.05	-	-	5	-
Glant swamp taro	548	-	0.9	-	-	-	31.0	-	334	56	1.2	-	-	0	0.05	0.07	0.88	-	-
Elephant yam	339	78.5	2.0	-	-	-	18.4	-	38	38	2.4	416	-	0	0.06	0.02	1.7	6	-
Taro leaves	255	81.4	4.0	-	-	-	11.9	-	162	69	1.0	963	-	5 535	0.13	0.34	1.5	63	163.0
Sweet potato tips	-	86.1	2.7	-	-	-	-	-	74	-	4	-	-	5 580	0.32	-	-	41	-
Banana	425	71.6	1.2	0.3	-	0.6	26.1	0.8	12	32	0.8	401	4	225	0.03	0.04	0.6	14	-
Plantain	476	68.2	0.9	0.2	-	0.4	29.7	1.0	19	38	0.6	-	-	475	0.15	0.06	0.7	11	-

Source: FAO (1972).

The nutritional composition varies from place to place depending on climate, the type of soil, the crop variety and other factors.

Apart from supplying energy and vitamin C, cassava roots are considered poor in nutritional terms. They have been implicated as a major cause of malnutrition in parts of Africa. However, the nutritive deficiencies of cassava need not be a cause for concern, when it is consumed with other supplementary foods such as meat, beans and leafy vegetables.

Nartey (1977) observed that cassava is produced and utilised as human food, predominantly in geographical areas where animal protein supply is inadequate. For that reason, diseases associated with protein deficiency such as kwashiorkor prevalent in those areas, cause many people to implicate cassava as the direct cause of malnutrition.

However, it has been suggested that, since diseases associated with protein malnutrition are invariably engendered by low intake of poor quality proteins, it is quite unjustified to consider the high carbohydrate content of cassava as being directly responsible for causing protein deficiency. At the same time, diets containing high ratios of cassava, lead to nitrogen imbalance, which can be alleviated by supplementary addition of essential amino acids, particularly methionine and tryptophan to the diet (Nartey, 1977). Cock (1985) stated that it is true that people who consume large quantities of cassava with little protein supplement will be malnourished.

The cassava problem, as related to "kwashiorkor disease", means that people with low incomes are prone to consume it in excessive quantities, because it supplies sufficient

calories and gives a feeling of satiety in their diets. Therefore many suffer from deficiency of protein and/or vitamins (Grace, 1977).

Apart from this, cassava still has the advantage of being cheap, meaning it can give required energy at a low cost. Cock (1985) concluded that increasing protein percentage in a diet deficient in total energy will not alleviate protein deficiency because, the body will be undernourished, and utilise protein in diet as an expensive energy source.

Increasing the carbohydrate supply in the diet can increase the protein utilisation efficiency. In general cassava can supply a cheap energy source, while other foods used are to supply the necessary protein, vitamins, minerals and fat. Mpoko Bokanga (1995) reported that cassava leaves are also a good source of protein, minerals and vitamins.

Cassava is normally subjected to treatments such as soaking in water, grating, grinding, fermenting, roasting, pounding etc. This causes a considerable loss of some elements and vitamins. Doku (1969) reported that almost all vitamins are lost when cassava is processed into <sup>1</sup>gari or <sup>2</sup>kokonte. Vitamin B<sub>1</sub>, vitamin B<sub>2</sub>, Niacin and vitamin C of gari and kokonte are lower than that of fresh cassava as shown in Table 2-2.

Doku (1969) explained how attempts were made in Nigeria to improve the nutritional value of gari by the addition of minerals and vitamins, as shown in Table 2-3.

It is further suggested that the nutritional value of cassava products such as kokonte flour, yake yake and tapioca could be improved on the same lines as fortified gari or

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<sup>1</sup> dried cassava product obtained by fermenting grated cassava roots.  
<sup>2</sup> Sun dried cassava root pieces.

**Table 2-2 Estimates of vitamins and mineral content of some cassava products (Milligrams per 100 grams product)**

	Vitamin B1	Vitamin B2	Niacin	Vitamin C	Calcium
Fresh cassava	0.05	0.04	0.06	34.0	26.0
Gari white	-	-	-	-	46.0
Gari yellow	-	-	-	-	83.0
Flour and meal	-	-	-	-	20.6
Roots dried after soaking (kokonte)	-	-	0.8	-	86.1
Cassava cakes	0.06	0.1	-	-	-
Boiled roots	-	-	-	20.33	-
Roasted roots	-	-	-	26.46	-

Source: Doku (1969).

**Table 2-3 The comparison of original white and fortified gari (dry weight basis)**

	Gari	Fortified Gari
Water (g)	8.5	7.3
Protein (g)	1.5	7.9
Fat (gm)	0.1	13.3
Carbohydrate (g)	87.0	77.9
Ca (mg)	45.6	73.3
P (mg)	56.9	140.9
Fe (mg)	2.2	3.3
Thiamin (mg)	0.12	0.24
Riboflavin (mg)	0.04	1.0
Vitamin A (I.U.)	0.0	16.0
Calories	338.0	341.0
Biological Value	47.0	54.0

Source: Doku (1969).

they could be taken with soups and stews of high nutritional value like beans. Kokonte are sun dried cassava root pieces. Yake yake refers to steam baked cassava cakes made from dried cassava meal and tapioca is the product made from roasted starch granules obtained from the liquid squeezed out of grated cassava roots (Doku, 1969).

### **Cyanogenic glucosides**

The presence of cyanogenic glucosides in the cassava root is a limiting factor in the use of cassava products for food (Nestle, 1973). Cassava is a cyanophoric plant meaning that it has the capacity to produce hydrogen cyanide. Cyanophoric plants contain cyanogenic glucosides, and the enzymes which hydrolyse them, in different compartments of their cells (Nartey, 1973). When cellular the structure is disrupted, the cyanogenic glucosides are hydrolysed by the enzyme.

The cyanogenic glucosides present in cassava are, linamarin, [2-( $\beta$ -D-glucopyranosyloxy)isobutyronitrile] and lotaustralin (methyl-linamarin) [2-( $\beta$ -glucopyranosyloxy)methylbutyronitrile], both of which are hydrolysed to hydrogen cyanide by endogenous linamarase (linamarin  $\beta$ -D-glucoside glucohydrolase) when the tissue gets damaged (Cooke, 1978). Linamarin and lotaustralin are bitter in taste and are soluble in dilute acids and cold water. They can slowly decompose to hydrogen cyanide, acetone and glucose in cold water, but boiling increases the rate of decomposition (Nartey, 1978). This may be why people refer to bitter cassava as toxic and sweet cassava non-toxic.

Figure 2-1 shows the structures of linamarin, lotaustralin and their metabolic precursors, valine and isoleucine.

### *Synthesis and Distribution*

Linamarin and lotaustralin, usually found in cassava in ratio of 10 to 1, are synthesised from amino acids valine and isoleucine respectively. Originally, it was thought that cassava produces cyanogenic glucoside in all its tissues, throughout the growth cycle, including sap exudates (Nartey, 1978). Recently, it has been established that the cyanogenic glucosides are only synthesised in leaves and translocated to all parts of the plant, where they accumulate (Koch *et al*, 1992).

De Bruijn (1971) found that there was a decrease of cyanogenic glucoside production in leaves with progressive ageing, and in young expanding leaves the cyanogenic glucosides content of the leaf petioles was much higher than the blades. He also reported that the apical portions of stem contain relatively high levels of cyanogenic glucosides which decrease towards the base.

Figure 2-2 Shows the metabolic pathway for synthesis of linamarin given by Koch *et al* (1992).

At the time of harvest of cassava roots, the amounts of cyanogenic glucosides vary from a few milligrams to 250 mg or more per kilogram of fresh roots (Grace, 1977). It has been observed that the cyanogenic glucoside content of the cassava plant is markedly increased by drought and by potassium deficiency.

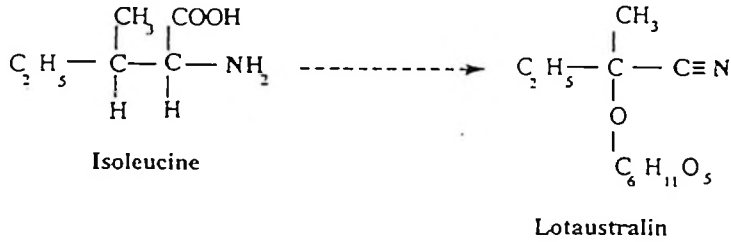
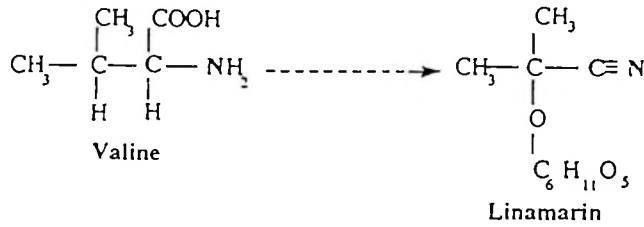


Figure 2-1 Linamarin and Lotaustralin and their metabolic precursors, valine and isoleucine

Source: Bokanga (1995).

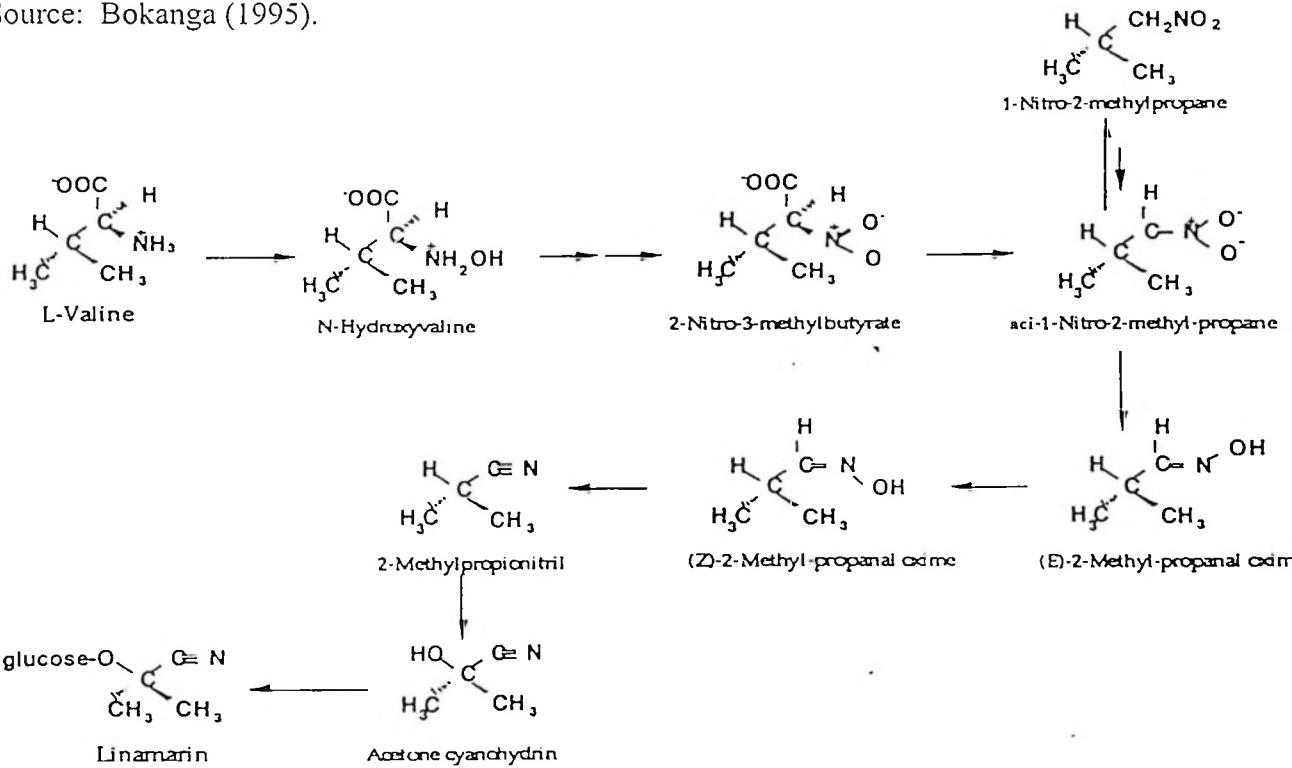


Figure 2-2 Biosynthetic Pathway of Linamarin in Cassava

Source: Koch *et al* (1992).

Although some varieties are referred to as "sweet" suggesting that do not contain cyanogenic glucosides, there are no completely cyanide- free cultivars found so far (Nartey, 1978).

### *Hydrolysis*

Linamarin and lotaustralin are stored inside the vacuoles in the cytoplasm, while the enzyme, linamarase is located on the cell wall (Mpkong *et al*, 1990). When the plant cells are intact, there is no contact between the cyanogenic glucosides and the enzyme. The enzyme can meet and act on the cyanogenic glucosides only when the tuber is cut or damaged. The action of linamarase on linamarin and lotaustralin is the hydrolytic release of acetone cyanohydrin and 2-butanone cyanohydrin respectively. This is shown in Figure 2-3 . Acetone cyanohydrin and 2-butanone cyanohydrin are acid-stable but hydrolyses spontaneously under neutral and alkaline conditions to liberate hydrogen cyanide (HCN) (Fomunyan *et al*, 1985). Hydrogen cyanide is a gas that is removed by volatilisation.

The glycosidic bonds of linamarin and lotaustralin can also be cleaved by hot dilute acids, to form D-glucose and the corresponding  $\alpha$  -hydroxynitriles which dissociate easily into a carbonyl compound and hydrogen cyanide when catalysed by a base (Nartey, 1978).

### **Cyanide Toxicity**

Cyanide is a deadly poison, which inhibits enzymes involved in ATP production in cells. Cyanide inhibits a wide range of enzymes, including metal- containing enzymes

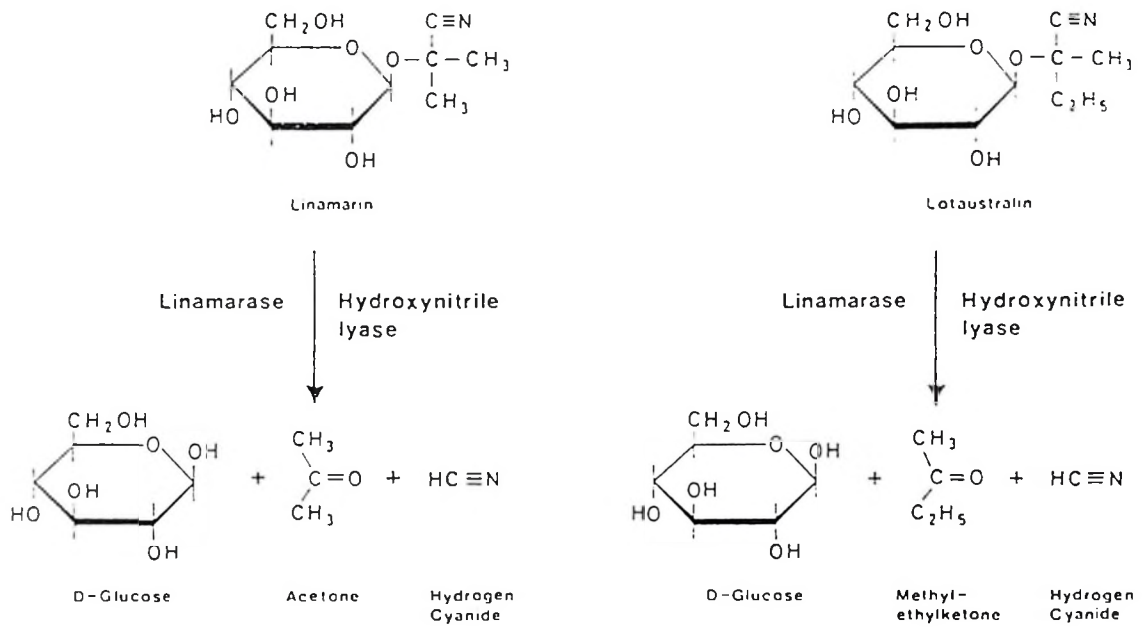


Figure 2-3 The hydrolytic products of the cyanogenic glucosides of cassava, Linamarin and Lotaustralin

Source: Nartey (1978).

and haemo proteins with which they react to form stable complexes. Dixon and Webb (1965) reported that terminal oxidase in the respiratory chain is susceptible to cyanide inhibition.

Cyanohydrins are the most dangerous form of the cyanogens. Cyanide can react with carbonyl compounds and reduce thiol group and disulphide bonds. Therefore, cyanide ingestion can cause serious physiological hazard (Nartey, 1978). In the blood stream, cyanide is converted to thiocyanate by the enzyme rhodanase. The thiocyanate as such is not harmful. It contains sulphur and is excreted in the urine. This leads to increased demand for sulphur containing amino acids. It also interferes with iodine uptake of the thyroid, resulting in goitre (Delange *et al*, 1973; Ekpechi, 1993).

According to Montgomery (1969) the lethal dose for hydrogen cyanide varies from 0.5 to 3.5 mg/kg body weight in humans. However, several millions of people living in the tropics consume cassava on a daily basis without any obvious sign of being poisoned (Bokanga, 1995). Although acute toxicity occurs very rarely, it can cause death in humans. This can result from the consumption of poorly prepared or completely unprocessed cassava roots (Nartey, 1978). Symptoms of acute cyanide poisoning include, abdominal pains, vomiting, mental confusion, muscular paralysis and respiratory stress as cyanide affects the cytochrome oxidase system, which may result into coma (Montgomery, 1969).

In general, in malnourished populations, some chronic effects of long term consumption of cassava include, endemic goitre, tropical ataxic neuropathy and cretinism (Cooke, 1983) and 'Konzo' disease (epidemic spastic paraparesis) (Mlingi *et al*, 1991). Tropical

ataxic neuropathy is a collective term for disorders which include, lesions in the skin, mucous membranes, optic and auditory as well as peripheral nerves (Osuntokun, 1973). Cretinism on the other hand is a condition of stunted growth in children.

However, in some areas in Latin America and Asia where cassava consumption is high, no cases of goitre and ataxic neuropathy have been reported (Cook, 1985). These people consume considerable quantities of animal and fish protein thus they have high levels of sulphur amino acids intake which play a part in cyanogenic glucoside detoxification.

### ***Uses of Cassava***

Cassava is grown chiefly as human food, but it is also an important animal feed, and it has several significant industrial uses. Apart from the economic activity inherent in cassava growing itself, the processing and marketing of cassava generate employment and provide opportunities for the development of rural industries (Cock, 1985). Being one of the cheapest sources of food energy, cassava could play a major role in meeting developing countries' rising consumption of both food and animal feed. Cock (1985) continued to explain that cassava can save developing countries from importing cereals, because it can grow well on land that is not suited to cereals and other food crops.

### **Utilisation as human food**

Cassava is extensively cultivated throughout the tropics for its starchy roots, which form a primary or secondary staple for many people (Coursey, 1973). The crop is an efficient producer of carbohydrate. It is tolerant to drought, grows fairly well in poor soils with low pH and is relatively resistant to diseases and insect pests. It has no precise maturity,

and can be left in the ground and harvested nearly any time of the year, thus being good security against famine (Lancaster *et al*, 1982).

Its potential yield is greater than for other crops, and production in calories per hectare is higher than any other staple food, while it requires minimum labour to produce (Weber *et al*, 1980). The consumption patterns of cassava differ among regions. In Africa, nearly all cassava is used for human consumption. In Asia, about half of the cassava is produced for direct human consumption, and the remainder is being exported in form of chips and pellets, while in Latin America, the amount of cassava produced for human food is only about 40%. More than half of cassava eaten is processed into forms such as flour, meals and starch (Cock, 1985).

According to Asiedu (1989) fresh peeled cassava is eaten as a vegetable after boiling or roasting. In West African countries, cassava is boiled and pounded with boiled plantain to form an elastic dough called fufu, which is consumed with vegetable and meat soups. The roots of the sweetest varieties are sometimes eaten raw as an (in -between- meals) snack or thirst quencher. When cooked, the roots become equivalent to potatoes, but their taste is considerably 'heavier.' In Indonesia the roots are often wrapped in leaves after covering them with a yeast preparation, then fermented to produce a soft and slightly alcoholic side-dish (Grace, (1977). Peeled cassava roots are also sliced, dried and ground into flour and processed into many different products.

### ***Traditional cassava products***

Cassava roots are processed into different food products. The products vary from one place to another.

## 1. Farinha de Mandioca

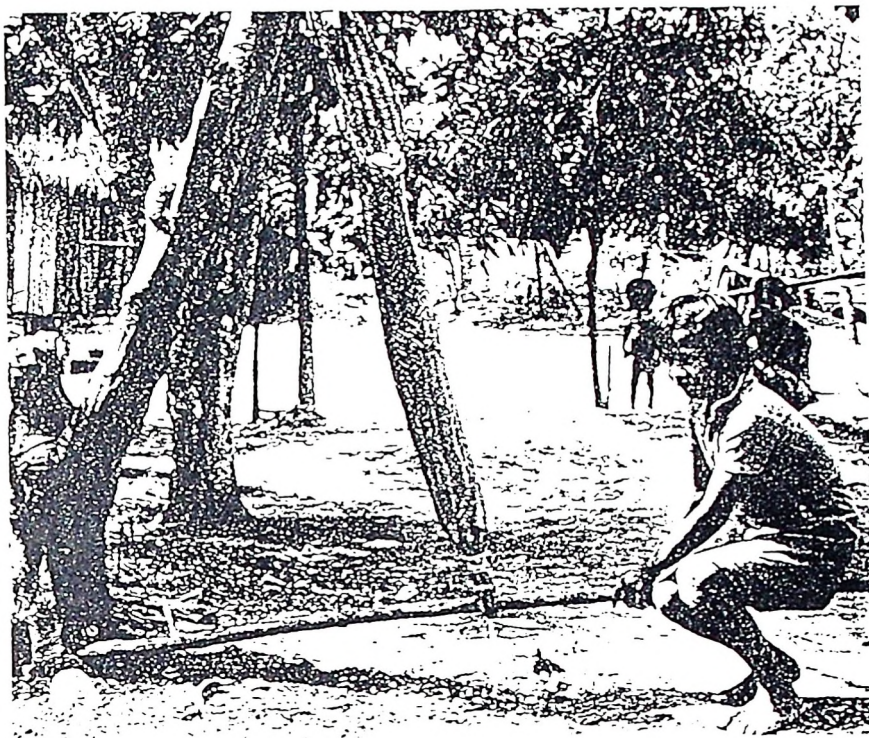
This type of food from cassava is popular in Brazil and other parts of South America. Asiedu (1989) explained the preparation of farinha de mandioca, which consists of peeling, grating, pressing, mixing, heating and roasting. After grating or rasping, the cassava pulp is packed into tipiti (a basket-weave tube). The tipiti Plate 2-1, as shown in, is stretched and its internal volume reduces, squeezing juice from the pulp. During this operation, the pulp is subjected to considerable pressure, causing the juice containing cyanogens to drain off. However, the tipiti is not used in large scale production practiced in factories.

The moist mash from the tipiti is mixed with 3-day old pre-fermented pulp, pounded together and rubbed through a sieve. Then the pulp is roasted in large flat pans. During roasting, the pulp is turned continuously with a wooden rake and results in a granular product. This is then eaten with meat and gravy. On the other hand, when the pulp is heated more intensively, without stirring to form a slightly brown mass, it forms Couac (bread) which is then sun dried and can be stored for a long time.

## 2. Gari

Gari is a popular food among the low-income groups in West Africa and Nigeria (Grace, 1977). It is produced by fermenting grated cassava roots, semi-dextrinizing the pulp by heat and then drying the product. The production of gari is a modification of the process for the manufacture of farinha de mandioca, but it has a characteristic flavour (Cock, 1985). Cassava roots are washed, peeled and grated, the resulting pulp

Plate 2-1 Tipiti



Source: Cock (1985).

is put into a cloth bag subjected to pressure by heaping stones and logs on the bag. The juice is squeezed out. The bag is left for about 2-4 days, and the pulp undergoes fermentation. This method is not considered to be as effective as the tipiti in squeezing out the juice. Later on the fermented pulp is removed from the bag, sieved and roasted or fried usually with palm oil in shallow metal pans or small pots until dry. Then the product is packed into bags and stored.

Cassava bacteria (*Corynebacterium manihot*) produce lactic acid and formic acid from starch during fermentation, which causes development of the characteristic flavour of gari. When the pH has fallen down to about 4.2, the fungus (*Geotricium candida*) produces more acid and the characteristic aroma.

### 3. Cassava rice (ladang)

Ladang is a popular food in Philippines and some other parts of South East Asia. According to Asiedu (1989) cassava rice is prepared by shredding the roots, and pressing the grated pulp in a cloth to remove the juice. The drained mass is whirled in a winnowing basket and forms pellets. The moisture content and the speed of winnowing determine the size of the pellets. Sifting gives pellets of more or less uniform size. They are then steamed or dried. In an alternative way as explained by Grace (1977) the roots are soaked in water in earthenware jars for five to seven days, until they are soft. Then they are macerated, fibre removed and the mass is air dried and made into pellets.

#### 4. Kokonte

It is common in West Africa. Doku (1969) explained kokonte preparation, whereby cassava roots are peeled, cut into pieces and dried in the sun, usually on roofs or along the road side. The dried roots (kokonte) are milled into flour. The flour is usually cooked by adding it to boiling water, and stirring until the correct consistency is obtained (sticky porridge).

#### 5. Chikwangu

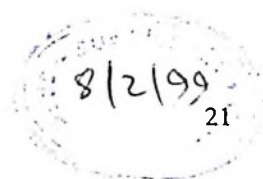
It is an African type of food. Cassava roots are soaked in water for several days until soft, then the roots are peeled and macerated. Fibres are removed and the dough formed is wrapped in banana leaves and boiled (Grace, 1977).

#### 6. Tapioca

This is obtained when starch is allowed to settle from the juice pressed out from the grated cassava pulp. The supernatant juice is decanted and the starch is washed, dried and roasted in a pan with continuous stirring like gari, but using low fire. The starch grains burst open and partial dextrinization of starch causes particles to adhere to form larger granules of various sizes (Doku, 1969).

#### 7. Fufu

Fufu is common in Sierra Leone. During its preparation, cassava roots are washed, peeled, grated, sieved and allowed to stand for sometime, but the juice is not squeezed



out. Fermentation takes place and at this stage, the juice is drained off. The resulting dough is stored and used to prepare fufu by putting some dough in a pot of boiling water and stirring continuously to produce a sticky meal.

#### 8. Yake yake

This is also common in West Africa. Its preparation involves peeling, washing and grating of cassava roots. The pulp is dried and sieved, then mixed with some salt and moulded into flat small 'cakes' which are steam baked for a few minutes. It is eaten with soup or stew.

#### 9. Casabe

This is an important product in the Caribbean islands and on the Atlantic coast of Colombia and Venezuela. Cassava is processed in the same way as for the production of farinha, except that the squeezed cassava mash is kneaded into a flat cake and baked.

#### **Cassava as animal feed**

Cassava is widely used in most tropical areas for feeding pigs, cattle, sheep and poultry. While in Brazil and Paraguay, animals are fed on considerable quantities of fresh cassava roots, in Asia, most cassava is dried before being used as animal feed (Cock, 1985).

Cock (1985) reported that pigs and poultry which were fed on cassava as an energy source, even with supplemental proteins, had lower growth rates and feed conversion efficiencies than animals on cereal - based diets. Recently it has been found that

methionine, plays a role in the detoxification of cyanogens. With the addition of protein, methionine and adequate levels of vitamins and minerals, low levels of cassava can replace maize in pig and poultry diets with no decrease in performance.

Cassava has become an important animal feed for a long time. A large quantity of cassava chips and pellets are exported to the European Community, mainly from Thailand, Malaysia and Indonesia. Cassava roots are an excellent energy source for ruminants, whether dried or, in case of sweet cassava, fresh (Cock, 1985).

Grace (1977) explained that chips is the form in which most dried cassava roots are marketed. These are dried irregular slices of roots, which vary in size but do not exceed 5 cm in length so that they can be stored in silos. Cassava chips processing starts with sorting and washing the roots. They are then shredded by machine, and cut into slices as they pass through the machine. The sliced roots are spread out to dry, where they are turned periodically to enhance drying. Artificial drying may be required in areas with high rainfall.

The pellets are obtained from dried and broken roots by grinding and moulding into a cylindrical shape. The cylinders are about 2-3 cm long and 0.4-0.8 cm in diameter and are uniform in appearance and texture. According to Grace (1977) pellets are nowadays preferred to chips, because their quality is more uniform, they also occupy less space than chips thus reduce transportation and storage costs.

Cassava can also be found in the form of meal. This is the powdery remains of chips and roots after processing to extract edible starch. The quality of the meal is low compared

to chips and pellets. Its starch content is low and it includes a lot of sand (Fullerton, 1929). But it is still used by some farmers for its cheapness.

### **Industrial Uses**

Cassava is also an important raw material in starch production. Starch from different cereals and tubers including cassava, is widely used in the food industries (Cock, 1985). Starch can be used in the form of cooked starch foods, e.g. custard. It can be used as a thickener in soups, baby foods and sauces. This is due to its paste formation properties. Grace (1977) explained the use of starch as the filler contributing to solid content of soups and ice cream. Starch can also be used as a binder especially in sausages and processed meats. This helps to consolidate the mass and stops it from drying out during cooking. Due to its high water-holding capacity, starch can be used as stabiliser especially in ice cream.

Starch is also used in the manufacture of candies and boiled sweets, in the making gums and pastes, also sprinkled on sweets to prevent them from sticking together. It is widely used in the manufacture of monosodium glutamate (MSG) in Far East and Latin America countries.

On fermentation by yeast, starch produces alcohol. Cassava contains 30% starch and 5% sugars. When dried, the roots have approximately 80% fermentable substances. Roots are washed, crushed into thin pulp and screened. Sulphuric acid is added to allow saccharification, when total sugars reach 15-17% of the content, fermentation by yeast is allowed to take place for about three to four days. Sodium carbonate is used to adjust the pH. Distillation is carried out to separate the alcohol. A ton of

cassava, can produce between 70-110 litres of absolute alcohol. This is mainly used for industrial purposes such as in cosmetics and solvents (Grace, 1977).

### **Non food uses**

Starch is widely used in the textile industry for sizing, cloth finishing and printing. In the paper industry, starch is used mainly to improve the finish, appearance, strength and printing properties. It also makes papers resistant to scuffing and folding (Whistler and Paschall, 1973). According to Grace (1977) cassava starch is used as a natural adhesive. It can be in the form of a roll-dried adhesive or a liquid adhesive. Starch is converted to dextrans by using chemicals, as well as enzymes. Cassava dextrans are preferred in remoistening gums for stamps and envelope flaps. It has good adhesive properties and acceptable taste and odour (Knight, 1969).

Dextrans are mainly used in the corrugated cardboard industry for the manufacture of cartons, boxes and various packaging materials (Kerr, 1968). Starch-based products, are used as adhesives for wallpaper and for various domestic uses. Again, starch is very useful in coating and binding sand grains, which are used in moulds in the manufacture of castings for metals.

## ***Cassava Processing***

### **Reasons for processing**

Cooking and processing of cassava offer some solution to two of its drawbacks, which are toxicity and perishability (Cock, 1985). Sometimes people eat raw cassava, (sweet varieties) as a snack but generally cassava is not consumed raw because of its toxicity.

Cassava cooking makes it palatable and digestible. Processing of cassava into dried products, reduces the levels of cyanogens in the final product, and extends the storage life of highly perishable cassava roots in their fresh condition (FAO, 1990).

### *Improvement of roots keeping quality*

Cassava roots are processed because, they are extremely perishable (Cock, 1985). Within two days of harvesting, cassava deterioration starts. This postharvest deterioration of cassava involves two separate processes. It starts with internal discoloration which spreads along the vascular system from wounds, then after a few days, microbial rotting follows (Booth, 1976; Rickard and Coursey, 1981).

The initial type of deterioration known as physiological (or primary) deterioration, starts within 24 hours after harvest, and is characterised by fine blue-black or brownish discoloration, (Vascular streakings) observed in cut or in damaged areas in the roots (Booth, 1976). Then follows microbial (secondary) deterioration which usually starts within a week after harvest. This is characterised by brown streaks throughout the fleshy part of the root and soft spots (Cock, 1985).

Marriot *et al* (1978) investigated the effect of storage environment on development of physiological deterioration, and observed that discoloration does not penetrate into the roots, when water loss from the cuts or sites of periderm injury is suppressed.

Booth (1976) suggested that wound healing or curing which occurs during storage at high temperature and high humidity, can prevent physiological deterioration. It was

further observed that suppression of moisture loss has an effect on root metabolism and on susceptibility to physiological deterioration, as well as on stimulation of wound healing.

In conclusion, Marriot *et al* (1979) said that physiological deterioration occurs due to rapid loss of water through sites of root injury. So, physiological deterioration can be suppressed by reducing water loss even without wound healing. However, wound healing is vital for suppression of microbial deterioration. The earlier symptom of physiological deterioration, the blue-black vascular discoloration, is a result of the formation of a pigmented material in the cells of the parenchyma adjacent to xylem vessels, and are passed into the vessels and occlude them (Rickard, 1979). The pigmented material consists of carbohydrates, lipids and lignin-like material. The more extensive discoloration of the storage parenchyma occurs due to the formation of phenolic compounds, including a coumarin derivative scopoletin (Rickard, 1989).

Lozano *et al* (1978) reported that cassava roots can be resistant to physiological deterioration when cassava plants are pruned to near ground level two or three weeks before harvesting.

#### Cassava roots storage

Cassava usually has to be consumed within a day or two due to lack of satisfactory long-term storage methods. The main approach to the technology of fresh cassava storage, is to develop storage structures or packaging systems which effectively inhibit water loss from cassava roots after harvesting (Coursey *et al*, 1982). The methods either prevent water loss by covering roots with impermeable barrier such as

plastic or soil, or the roots are packed into material which retains moisture, such as wet sawdust.

Some people apply paraffin wax to coat cassava roots, to exclude oxygen from the wounded surfaces of the roots (de Buckle *et al*, 1973; Zapata and Riveras, 1978). The most effective, reliable and flexible system developed so far consists of packing roots into sawdust, coir dust or similar absorbent material moistened with water (Booth, 1977). This method has been found to work well in Colombia, India, Jamaica and Fiji (Marriot *et al*, 1974; Aiyer *et al*, 1978; Sivan, 1979). According to Aiyer *et al* (1978) these methods have been successful in reducing losses to a low level for a storage period of up to four weeks. But with time, the absorbent packing material dries out.

Lozano *et al* (1978) reported the use of polythene film as a liner inside the sacks for cassava storage. Within the period of two weeks, the method controls physiological deterioration effectively. Also polythene material can be used as sheets to cover cassava stored in bulk awaiting processing. Extended periods of storage require the use of a fungicide to control microbial spoilage.

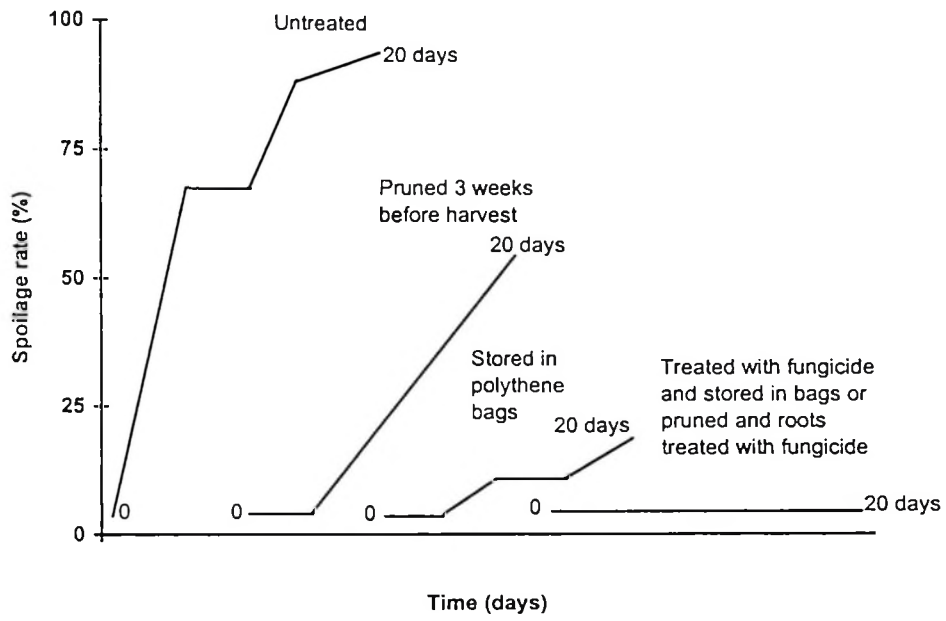
Cock (1985) reported that in Trinidad and Tobago, roots packed in polythene bags could be stored for up to two months. But, when the method was tried in Colombia, only physiological deterioration was prevented, the microbial spoilage caused

deterioration after 1 or 2 weeks of storage. The combination of fungicide and packing the roots in polythene bags retard spoilage for up to 3 weeks, as shown in Figure 2-4.

Fungicide is applied to the surface of the roots and is not translocated. Since the roots are always peeled before cooking, the fungicide residues are not likely to be a problem. Again, the fungicide Mertect (thiobendazole) normally used has low human toxicity (Cock, 1985). The whole process may look laborious, but in the existing high marketing margins, it could be economically feasible.

A clamp method, based on storage structures for potato has been tried also. Cassava roots are stacked onto a bed of straw, then covered with straw and sand (Booth, 1976). The system works, retarding spoilage by up to four weeks, but it is not possible to inspect the roots during storage. During the storage period, composition of free sugar increases from 5% to 15% dry weight, with a comparable loss of starch. But the roots are still acceptable organoleptically. Booth (1974) showed that the critical period for initiation of physiological deterioration is 24 hours after harvesting. Thus, it is essential to apply methods for retarding moisture loss immediately after harvesting.

With pruning, even severely damaged roots do not show physiological deterioration after harvesting. But, pruning causes changes in the roots that lower the eating quality and lengthen the cooking time. Cock (1985) reported that pruning permits cassava roots to be held for about five days before microbial deterioration begins. Freezing can prevent cassava spoilage but is very expensive. This method is applied on a very small scale in the Dominican Republic and Costa Rica for export to large urban centres (Cock, 1985).



**Figure 2-4 Root spoilage**

Source: Cock (1985).

Traditional methods used in Tanzania include, burying the cassava roots in the ground, covering them with soil. Another method used is to cut both ends of the roots and apply ash. The roots can be stored up to 2 weeks. Sometimes, especially at market places, cassava roots are packed into empty fertiliser sacks and moistened with water from time to time.

### *Detoxification*

Toxic varieties of cassava continue to be grown due to their agricultural advantages, (draught and poor soils tolerance) and a variety of processing techniques employed reduce cyanogenic glucosides to low levels and produce foods which are safe for consumption (Jones *et al*, 1994).

Such technologies are based on disintegration of cassava roots either by mechanical disruption (grating) or by fermentation (wet and solid-state), followed by drying.

Ayernor (1985) reported that soaking and crushing of cassava roots followed by sun drying results in removal of cyanogenic glucosides up to 98.6%, and 95% respectively. Nambisan and Sundaresan (1985) investigated the effect of other processing methods on cyanogenic glucosides content of cassava. They found that baking, frying and steaming of cassava roots removed about 20% of cyanogenic glucosides only, while sun drying of chips removed between 40-70% depending on the chip size.

During the fermentation process, accumulation of acid lowers the pH (around 4.0) and this inhibits linamarase function, thus slowing down cyanogenic glucosides hydrolysis

and cyanohydrins breakdown. Sokari and Karibo (1992) suggested the possibility of manipulating processing parameters such as water content and temperature to achieve rapid detoxification of the pulp before the onset of fermentation. So in general it was concluded that the important stages in gari preparation with regard to detoxification are grating or mincing of roots before fermentation and the final roasting (garification) (Vasconcelos *et al*, 1990).

On the other hand, Cooke and Maduagwu (1978) and Oke (1983) argued that endogenous linamarase is not sufficient to remove all cyanogenic glucosides in cassava roots. For that matter, traditionally processed cassava foods always contain some residual cyanogens.

### **Processing Methods**

The methods of processing and cooking range from simple boiling to elaborate fermentation, drying and grinding to make flour. The simplest way of preparing cassava is to peel it immediately after harvest and boil it for 10-40 minutes until soft. This may be eaten or may be cut into long chunks and deep fried. However, sweet cassava must be used (Cock, 1985). Cooked cassava looks like potato but its taste is considered 'heavier'. Cyanogenic glucosides are destroyed slowly by cooking the sliced roots, starting with ample cold water, so that gradual heating hydrolyses the toxic principles. Sometimes cassava roots can be roasted in hot ashes.

On the other hand, the heat of boiling and the loss of water inactivate the enzymes, and thereby reduce the degree of hydrolysis of cyanogenic glucosides by linamarase

(Nambisan and Sundaresan, 1985). So it is not suitable for cassava roots with high levels of cyanogenic glucoside (bitter varieties).

### *Fermentation*

Most cassava foods utilised in Africa are fermented products (Westby, 1994). The types of fermentation practised are, wet fermentation and solid-state fermentation. In wet fermentation or submerged fermentation, cassava roots, whole or in large pieces are steeped in water for 3-5 days (Bokanga, 1995). Solid-state fermentation comprises of mash-fermentation and low-moisture fermentation. In mash-fermentation, the pulp is obtained by grating or rasping fresh cassava roots and left to ferment in a container for several days (Westby, 1994). In the case of low-moisture fermentation, peeled cassava roots are heaped together and mould growth allowed to develop at the surface of the roots. It is purported that the mould growth assists in reducing the cyanogenic glucosides content of bitter cassava varieties (Esser and Nout, 1989; Esser, 1992).

Bokanga (1995) stated that nearly all fermentations rely on the fortuitous presence of microbes on the roots/or in the water, and on the prevailing favourable conditions for production of desired product. Since there is no control of the fermentation process, the flavour, aroma and texture of the fermented product varies with season, location and producer.

Several researchers have identified the micro-organisms associated with wet fermentation and mash-fermentation of cassava (Abe and Lindsay, 1978; Ngaba and Lee, 1979; Okafor *et al*, 1984; Nwankwo *et al*, 1989). The most important micro-organisms in these types of fermentation are, Lactic acid bacteria, including

*Lactobacillus plantarum*, *Streptococcus faecium* and *Leuconostoc mensesenteroides*. They produce acids that characterise cassava fermentation. In general many different microbial species have been isolated during production of cassava fermented products.

Retting which is softening of plant materials immersed in water occurs during wet fermentation. It is due to the breakdown of the materials that give the plant cells their integrity. In cassava roots, the cell wall is made up of cellulose held together by pectin (Grace, 1971). Retting is normally caused by *Bacillus* sp. micro-organisms, especially *Bacillus corynebacterium*. *Bacillus* sp. also play an important role in the starch hydrolysis and acid production in cassava fermentation (Collard and Levi, 1959; Okafor, 1977; Okoli, 1979; Eijiofor and Okafor, 1981).

It has been reported that wet fermentation is the most efficient process for reducing levels of cyanogens in cassava, up to the rate of 95-100% (Ayernor, 1985; Ezeala and Okoro, 1986). Several factors have been suggested to be responsible for the removal of cyanogens from cassava during wet fermentation. One factor could be the changes in texture of the plant tissues, which allow the cyanogenic glucosides to be released from the vacuole, and come into contact with linamarase enzyme from the cell wall membrane. In that case the cyanogenic glucosides can be hydrolysed or leached out. Another factor is said to be the increase in  $\beta$ -glucosidase activity in cassava tissue and the possibility of fermentation micro-organisms to utilise the cyanogenic glucosides and their break down products.

Vasconcelos *et al* (1990) indicated that detoxification of cassava in mash-fermentation has a different mechanism. Grating disrupts the structural integrity of plant cells, allowing the cyanogenic glucosides from the vacuoles to come into contact with

linamarase on the cell wall. So the following fermentation, has very little effect to the breakdown of cyanogenic glucosides.

### *Drying*

Drying is the oldest method of food preservation practiced by man (Brennan, 1994). There are two principal methods for cassava drying i.e. sun drying (natural) which depends on solar heat and mechanical (forced convection) drying which involves the use of hot air produced by combustion of fuel or some other heating operations (Steghart and Wholey, 1982; Fish and Trim, 1993). Various systems for drying solids in heated air include: kiln drier, cabinet drier, conveyor drier, bin drier, pneumatic drier and rotary drier. For economic reasons, sun drying is the main technique used with cassava (Coursey, 1982).

Several factors have been reported to govern the rate of sun drying of cassava which include, the chip size, loading density, initial moisture content, ambient conditions and type of drying surface such as concrete floors, (black painted or plain), bamboo or woven mats and perforated trays. It has also been explained that the rate of mechanical drying also depends on the chip size, loading density and initial moisture content, but will also be governed by the drying air condition and flow rate (Coursey, 1982; Steghart and Wholey, 1982; Fish and Trim, 1993). Results by Thanh and Lohan (1978) showed that the chip size and shape are very important in cassava sun drying. It was observed that strips dry quicker than slices. It was also observed that the black painted concrete achieved a greater reduction in drying time compared to ordinary concrete surfaces. For fast and uniform drying, the chips are spread in rows and turned and respread at

intervals during the day. The use of drying trays gives greater outputs than concrete floor because of improved air flow around the chips (Best, 1979).

Drying prolongs the shelf life of the food material. It reduces the water activity ( $a_w$ ) of the food to a value which will inhibit the growth and development of pathogenic and spoilage micro-organisms, and reduces the enzyme activity. Drying also reduces the food weight, making storage and transportation easier. However, it can cause undesirable changes in foods. Shrinkage can occur in solid food pieces changing their size and shape. Reconstituted products may not return to their original shape and size, and colour changes can occur during drying, especially when high temperatures are used (Brennan, 1994).

In cassava roots, drying reduces cyanogens, the rate of degradation depending on chip size, the presence or absence of peel and water activity (Jones *et al*, 1994). Cooke and Maduagwu (1978) noted that the rate of breakdown of cyanogenic glucosides determines the residual cyanogens concentration. Free cyanide is easily removed by drying but degradation of cyanogenic glucosides depend on the level of tissue damage and the length of time that linamarase remains active. Fish and Trim (1993) concluded that although sun drying is relatively cheap, it does not give product of high quality, may also have high final moisture content and can allow mould growth to develop during inclement weather.

### ***Mycotoxins in Cassava Products***

When cassava roots are processed traditionally, especially by solid-state (low-moisture) fermentation, and sun drying, proliferation of micro-organisms (fungi) on cassava takes

place. These include spoilage and toxinogenic micro-organisms, some of which may produce mycotoxins. Fungi can also grow on cassava when the products are poorly stored (Clerk and Courie, 1968; Esser and Nout, 1989).

Mycotoxins are extracellular zootoxic metabolites (exotoxins) produced by filamentous fungi (moulds) in foods consumed by man and animals. The diseases resulting from consuming food-borne toxins are known as mycotoxicoses (Moreau 1979). In general, mycotoxins include also antibiotics, such as penicillin and streptomycin, but the term 'antibiotics' is normally used for compounds produced by microbes that are toxic to other microbes (Mirocha *et al*, 1969). Mycotoxins can be formed in almost any organic material or product, subject to invasion and decay by moulds. However, mycotoxins are much more likely to be formed in some grains, seeds and plant products than others and are much more likely to occur under some circumstances than others. Soares and Rodriguez-Amaya (1985) explained that the mycotoxin problem is more serious in developing countries where climatic conditions and agricultural and storage practices are considered conducive to mould growth and toxin production.

According to Northolt and Bullerman (1982) the environmental conditions which influence mould growth and mycotoxin production are; water activity, ( $a_w$ ) temperature, pH and composition of the atmosphere depending on the type of growth. They reported the optimum temperature range for aflatoxins production by *A. flavus* as 25-30°C, and RH greater than 80%. The water activity ( $a_w$ ) has taken the place of moisture content as the most useful expression of the availability of water for growth of micro-organisms. Water activity is defined as a ratio of the vapour pressure of the product to that of pure water. The lower the  $a_w$ , the less moisture is available to the micro-organisms.

Most species known to produce mycotoxins have been reported to be in the genera, *Aspergillus*, *Penicillium* and *Fusarium* (Christensen *et al*, 1968). Toxigenic moulds occur everywhere. They are easily disseminated through their vegetative spores, which are produced in large numbers. Contamination of agricultural products with these moulds can occur before harvest (Northolt and Bullerman, 1982). Mould infection development and toxin production can occur in field or/and in storage. Field moulds include *Alternaria*, *Fusarium* and *Cladosporium*. Storage moulds include *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor* (Miller and Trenholm, 1994).

*Penicillium* sp. is of great economic concern, as the cause of food spoilage. It is more diverse in terms of species and range of habitats in which it can survive than other species. Mycotoxins produced by *Penicillium* sp. include bevirnamide A, citreoviridin, citrinin, cyclopiazonic acid, fumitremorgin B, gresofulvin, ochratoxin A, patulin, penicillic acid etc. (El-Banana *et al*, 1987).

*Aspergillus* is more abundant in the tropics (Krogh 1987). Species can grow at low  $a_w$  (Moss, 1987). They produce aflatoxins, sterigmatocystin, versicolorins and ochratoxins (Frisland and Samson, 1991). Minimum water activity for most storage moulds is 0.70 (Lacey, 1989). Adequate water for mould growth in grains may result from inadequate drying before storage, penetration of rain into storage structures or moisture migration because of temperature gradient within the storage containers (Christensen and Kaufman, 1974; Saver, 1978). Differences in temperature between portions of a grain bulk, can result in fairly rapid transfer of water vapour from warm to cool areas.

Moulds are capable of growing on various substrates. Substrates differ in their ability to

support mycotoxin production (Madhayastha *et al*, 1990), affecting both the nature and amount of metabolic toxin produced.

### **Types of mycotoxins**

The types of mycotoxins which has been reported to be found in cassava products are, aflatoxins, ochratoxins, zearalenone and patulin ( Andekunle and Ayeni, 1974; Gerona, 1986; Pohland and Wood, 1987; Esser and Nout, 1989; Jonsyn, 1989).

#### ***1. Aflatoxins***

Aflatoxins are produced by *Aspergillus parasiticus* and *Aspergillus flavus*. There are four types of aflatoxins, B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. *A. flavus* can only produce aflatoxins B<sub>1</sub> and B<sub>2</sub> while *A. parasiticus* can produce all four types of aflatoxins (Moreau, 1979). Aflatoxin B<sub>1</sub>, (most abundant of the derivatives) is a carcinogen, teratogen and considered of prime importance in public and animal health. Under ultraviolet light (about 365 nm), aflatoxins B<sub>1</sub> and B<sub>2</sub> fluoresce blue and G<sub>1</sub> and G<sub>2</sub> fluoresce green. They are slightly soluble in water, insoluble in non polar solvents and readily soluble in polar solvents such as methanol, chloroform and dimethylsulphoxide.

Cassava roots are supposed to be processed immediately after harvest otherwise, a coumarin compound known as scopoletin tends to accumulate in the roots. This compound fluoresces blue in UV light and has a similar RF value to aflatoxins B<sub>1</sub> in some TLC systems (Wheatley, 1984). Scopoletin interference has caused some very high aflatoxin values to be published for example Mota and Lourenco (1974) reported

cassava flour to contain aflatoxin B<sub>1</sub> between 500-4600 mg.kg<sup>-1</sup> and 1600 mg.kg<sup>-1</sup> in gari, also Masimango *et al* (1977) reported greater than 1000 mg.kg<sup>-1</sup> in flour (Bulatao-Jayme *et al*, 1982; Emerole *et al*, 1982; Wheatley, 1984; Whitley and Cock, 1985; Wareing and Westby, 1993).

The temperature for aflatoxin production ranges between 12°C-42°C with optimum temperature at 27°C-30°C (Davis and Diener, 1970). Noltholt and Bullerman (1982) observed that maintenance of low temperatures prevents aflatoxin production in stored products. But, other toxins like penicillic acid, patulin, zearalenone and T-2 toxin may be produced even at low temperatures. It has been observed that production of aflatoxin by *Aspergillus flavus* in mixed cultures with *Aspergillus niger* is less than in pure culture. Mixtures of moulds growing in grains and nuts in competition with *A. flavus*, seem to prevent aflatoxin production. Aflatoxins have been found in most staple foods such as cereal grains, nuts etc. (Stolof, 1976). Control of aflatoxin contamination requires the reduction of contamination in food and feedstuffs to harmless levels. Lillehoj and Hasseltine (1979) suggested establishment of good harvesting, handling and storage procedures to prevent formation of aflatoxin.

Aflatoxins induce both acute and chronic toxicity in mammals and other animals. These compounds act as carcinogens and teratogens as well as mutagens (Legator, 1969). Newberne and Butler (1969) and Hayes *et al* (1977) suggested that the effect of aflatoxin exposure may be acute or chronic depending on dosage and frequency of exposure. Sensitivity towards aflatoxins varies with species, age and sex of animals as well as the composition of the diet.

Aflatoxin B<sub>1</sub> is the most toxic followed by G<sub>1</sub>, B<sub>2</sub> and G<sub>2</sub> (Carnagham *et al*, 1963; Wogan, 1969; Wogan *et al*, 1971). It has been found that lethal doses (LD<sub>50</sub>) of aflatoxin B<sub>1</sub> is 0.335 and aflatoxin G<sub>1</sub> 0.78 mg/kg body weight in ducklings, and 9.0 mg/kg in mice (Butler, 1974). Moreau (1979) reported that acute toxicity of aflatoxins causes death of animals, causing the liver to become pale and discoloured, it also increases in size. Where chronic toxicity occur, there is loss of appetite and reduction in growth in animals. The liver is also affected with congestion and haemorrhagic or necrotic zones caused by the toxin (Philip, 1964).

Animal studies have shown proliferation of epithelial cell of the bile duct following aflatoxins ingestion (Newberne and Butler, 1969). It has been observed that just a few days before death, animals show characteristics of toxicity like staggering, nervousness and muscular spasms. Acute aflatoxin poisoning causes hepatic necrosis, derangement of hepatic functions, coagulopathy and extensive haemorrhagic lesions that frequently lead to death of farm animals (Madharan and Suryanarayana, 1967). Chronic aflatoxicosis causes changes in the liver, enlargement of gall bladder and changes in the bile duct epithelium. Lancaster *et al* (1961) provided evidence that aflatoxin is carcinogenic, after rats fed with toxin developed multiple tumours and lung metastases.

Aflatoxin B<sub>1</sub> has been reported to be a biosynthetic inhibitor. Large doses cause total inhibition and lower doses progressively affect different systems (Lijinsky *et al*, 1970). Clifford and Rees (1967) showed that aflatoxin B<sub>1</sub> interacts with DNA and inhibits polymerases which are responsible for DNA and RNA synthesis. It also suppresses DNA synthesis, inhibit mRNA and reduces protein biosynthesis. In humans, studies carried out in Kenya (Peers and Linsell, 1973), Mozambique (Prates and Torres, 1965; Van Rensburg *et al*, 1974) and Swaziland (Keen and Martin, 1971) correlated the

incidences of primary liver cancer with consumption of aflatoxins. Similar correlations were also established in Thailand (Shank, 1977) and Uganda (Alpert *et al*, 1971; Shank *et al*, 1972). Besides liver cancer, aflatoxins can also induce other abnormalities in human beings. Several workers suggested that aflatoxin induces Reye's syndrome in human beings (Shank *et al*, 1971; Bourgeois *et al*, 1971; Van Rensburg, 1977; Ryan *et al*, 1979; Nelson *et al*, 1980). But, later it was concluded that aflatoxin B<sub>1</sub> is probably not an important etiological agent of the Reye's disease after the detection of aflatoxin in only 1 of 12 liver specimens from children who died from the Reye's disease (Mullen, 1978; Ciegler *et al*, 1981; Busby and Wogan, 1984; Rogan *et al*, 1985).

## 2. Zearalenone

This type of toxin is produced mainly by *Fusarium roseum*. It is an estrogenic secondary metabolite. Mirocha *et al* (1969) reported zearalenone production by *F. moniliforme*, although in small amounts. It has been reported that rice is the best substrate for zearalenone production followed by corn and wheat. Zearalenone fluoresces blue-green when exposed to ultraviolet light (360 nm) (Holder *et al*, (1977); Ware and Thorpe, 1978).

Zearalenone is mainly known to affect swine, causing morphological changes and functional disorders of reproductive tract leading to retention of corpora. Other animals affected are chicken, turkey, monkeys and lambs. Lovelace and Nyathi (1977) reported the presence of zearalenone in Zambian home-brewed and commercial maize beer. Sherwood and Peberdy (1972) have stated that high moisture content and temperature between 12-14°C are necessary for the production of high levels of zearalenone.

### **3. Ochratoxins**

Ochratoxins have been reported to be found in swine feed in Denmark, Sweden and Ireland. Small amounts were found in peanuts, dried beans and mixed animal feeds. Ochratoxin A is produced by *Aspergillus ochraceus* and *Penicillium viridicatum* (Scott *et al*, 1972).

Krogh *et al* (1976 a,b) said that the main target organs for ochratoxins are liver and kidney. They cause tubular necrosis of the kidney, mild degeneration of the liver and enteritis of the small intestine. Ochratoxin A affects mainly the liver and kidney (Krogh 1974). It causes resorption of foeti in pregnant rats and teratogenesis and renal disfunction (Still *et al*, 1971; Brown *et al*, 1976). suggested that acute toxicity of ochratoxin A in ruminants is not likely because it occurs in small amounts naturally in feedstuffs. Toxic effects are more likely to occur in chronic low-level intoxication. It was suggested that the toxin cannot be transmitted into milk and urine in cows because it is hydrolysed in the rumen.

### **4. Patulin.**

Patulin is a carcinogenic lactone, found naturally in apple juice and a metabolite of *Penicillium* and *Aspergillus*. The main species which produces this toxin is *P. expansam* (Scott, 1974; Holder *et al*, 1977).

Apart from apple juice, patulin can be found in bananas, pineapples, grapes, peaches etc. Stinson *et al* (1978) observed that patulin is not stable during fermentation, so almost 99% is eliminated during alcohol fermentation. Ciegler *et al* (1976a; 1977) reported that

patulin is teratogenic. They further reviewed the acute and chronic toxicity of patulin and found that patulin is quite toxic to laboratory animals when injected subcutaneously or intravenously but not in oral feeding studies.

### ***Production of Cassava Flakes***

Cassava is a high moisture content, bulky crop. The lack of suitable preservation methods, is the main problem which makes it very difficult to ensure its supply throughout the year (Onayemi and Potter, 1974).

Preparation of cassava flakes, will provide a convenience food, and become a substitute for fresh cassava. Walker (1985) defined convenience foods as those products, in a preserved state, whether frozen, dehydrated or canned, which their preparation by the customer, require minimal skills and time to produce an acceptable dietary item.

No work has been done on production of flakes from cassava, however, there is substantial information on the production of potato flakes (Feustel *et al*, 1964; Sullivan *et al*, 1985; Wang *et al*, 1992) and sweet potatoes flakes (*Ipomoea batatas*) (Hoover and Harmon, 1967; Sparado *et al*, 1967; Walter and Purcell, 1978). Some work has also been done on production of yam flakes (Rodriguez-Soza, 1972a; Mohamed , 1973).

Cassava is a tuberous starchy vegetable, and is like potato in many aspects, therefore, the technology for production of potato flakes can be applied for cassava flakes production.

Flakes can be defined as a form of dehydrated mash of a particular food. They rehydrate

instantly to produce the mash with almost the same textural characteristics or 'feel in the mouth' as the mash prepared from the cooked fresh food.

Flakes preparation from roots involves, peeling, slicing, pre-cooking, cooling, cooking, mashing followed by drying on a drum drier (Van Arsdel *et al*, 1973).

According to Knaak (1976) the operating conditions which yield potato flakes of improved overall quality are, pre-cooking in water for about 20 minutes at 71°C, cooling for about 20 minutes and cooking for about 30-50 minutes. The suggested steam pressure of the drum is between 520-550 kNm<sup>-2</sup> (264-279°C drying temperature) and drum speed of 2 r.p.m.(2 seconds residence time), which depends on the size of the drum.

Cooking causes rupturing of starch cells, as they become hydrated, swollen and gelatinised (Ooraikul *et al*, 1974; Leelavathi *et al*, 1987). Starch gelatinisation is accompanied by an extensive leaching of low molecular weight amylose, which becomes responsible for stickiness, pastiness or glueyness of the reconstituted product (Ferrero *et al*, 1993).

Although the effect brought by gelatinised starch in flakes is not desirable, pregelatinised starch is widely used in different foods like, 'instant' puddings and pie fillings as thickening or gelling agents. Gelatinisation also improves the flavour of cooked cereals as it makes the grain starches lose that raw cereal flavour which is not desirable (Sullivan *et al*, 1961). The extent of gelatinisation in baked goods affects the properties of the product, its storage behaviour and its rate of digestibility.

Pre-cooking and cooling of the roots during flakes preparation, minimise the stickiness of the reconstituted mash by allowing starch retrogradation, whereby the starch molecules realign and associate by hydrogen bonding, thus decreasing their solubility (Sullivan *et al*, 1961). It was observed that on cooling starch paste, the molecules become less soluble and tend to aggregate and partially crystallise. However, retrogradation on the other hand can be undesirable, e.g. in bread staling, it also cause textural changes of some food containing starch like, canned soups, peas and meat preparation. (Collison, 1968).

In order to minimise cell breakage during mashing, this process should be carried out while the cooked roots are still hot, because the slices are still soft so mashing causes less damage to cells. When preparing yam flakes, Gooding (1972) observed that a waring blender produced a very sticky mash, with a large number of broken cells. He found the mechanical mixer to produce a sticky mash, while hand mashing caused no cell breakage at all.

Additives which includes sulphites, antioxidants, and emulsifiers are incorporated into the mashed material before it is dried, to improve flavour, stability and texture of the product. Sodium sulphite, sodium bisulphite and antioxidants prevent oxidative changes during processing and improve the shelf life. Oxidative changes cause losses of vitamin C during flakes preparation, which ranges between 30-100%. Oxidative changes also result in development of hay-like off-flavour during storage of the flakes (Van Arsdel *et al*, 1973; Sullivan *et al*, 1985).

Antioxidants commonly used are, t-butyl-hydroxyquinone (TBHQ), butyl hydroxyanisole (BHA), butyl hydroxytoluene (BHT) and propyl gallate (PG) (Wang *et*

*al*, 1992). These antioxidants are used in other food products e.g. TBHQ can be used alone in snack food and edible fats. It can also be combined with BHA and BHT. Citric acid in propylene glycol is also used to retard oxidation (Anon, 1973).

Another important cause of deterioration in dried foods which limit their storageability is browning. Browning can be enzymic or non-enzymic (Bolin *et al*, 1985). The flakes preparation is mainly affected by non-enzymic browning (Maillard reaction) in which the reaction occurs between carbonyl groups from reducing sugars and free amino groups from protein (Van Arsdel *et al*, 1973; Hurrell and Carpenter, 1977).

When lysine reacts with reducing sugars under mild conditions, they form a biologically unavailable lysine-sugar complex, which can lead to total destruction of lysine molecule on further heating (Finnot, 1973; Szczodrak, and Pomeranz, (1992).

During storage of flakes, there is a fall in levels of available lysine with little changes in the overall digestibility of protein (Hurrell and Carpenter, 1977). Sulphites control enzymic browning during flakes preparation by combining with reactive intermediates, and also they have a bleaching effect on the final colour (Van Arsdel, *et al*, 1973).

Non-enzymic browning is greatly influenced by the pH, temperature, and moisture content of the material. In general, the reaction is inhibited by low pH, low temperature, very low and very high moisture content. Maximum browning occurs between 0.5-0.7  $a_w$  (Lingert, 1990).

Some reports have indicated that vitamins including vitamin C together with other water soluble vitamins are lost due to leaching during the pre-cooking and cooking stages.

Kompel *et al* (1982) reported significant losses of thiamine (B<sub>1</sub>), riboflavin (B<sub>2</sub>), niacin and vitamin C during pre-cooking of potatoes. Further losses of niacin and vitamin C occurred during the cooling stage. He also pointed out that vitamin C losses during cooking are probably caused by a combination of leaching, thermal degradation and oxidation. Drum drying also caused vitamin C losses.

Reconstituted flakes mash becomes sticky, so emulsifiers are normally added to improve their textural characteristics. In food technology, emulsifiers are often added to improve the rheological quality as well as the stability and storage properties of many food products (Krog, 1971). In food products rich in starch, the effect of emulsifiers like saturated monoglycerides is often attributed to their ability to form insoluble complexes with amylose (Hoover, and Hadziyev, 1981b).

In general, emulsifiers fulfil three major roles: starch complexing/processing aid, emulsification and foam stabilisation. Emulsifiers such as distilled monoglycerides (GMS 900/ GMS 902) or sodium stearoyl-2-lactylate (SS2L) help to prevent the gel sticking to the equipment during processing. They also aid the rehydration of such products as dehydrated potato by controlling gelation and stickiness. In cake mixes, product such as sorbitan monostearate, SS2L and sorbitan ester/polysorbate blend (vykamol 83 G) are used to give greater volume and finer crumb texture to the cake, by their protein complexing properties. Emulsifiers also are used to emulsify fat in fat/oil-water systems, also as foam stabilisers in products like creams, spray-dried instant desserts and toppings (Dickison *et al*, 1989).

Proteins are also used as emulsifiers and stabilisers. This is due to their ability to interact with water, small ions and other polymers at oil/water interface. In that case, compounds

like sodium caseinate, potassium caseinate and skim milk powder are very effective emulsifiers, stabilisers and foaming agents (Walstra, 1988; Kim *et al*, 1996).

### **Drum driers.**

A drum drier consists of a revolving drum that is heated internally. The heating medium which is steam or water is circulated in the drum. The drum rotates about the symmetrical axis and is controlled with a variable speed drive. The material to be dried is applied as a thin uniform layer to the outer surface of the revolving hot drum surface, by using a feeding device. The dried material is scraped off the drum surface by the doctor knife. The drum driers can be single, double or twin drum units (Van Arsdel *et al*, 1973; Brennan, 1994).

Drum driers are flexible in their operations and their variables can be changed independently without affecting others. These includes, steam pressure which controls the temperature of the drum, rotational speed of the drum, measured as rotation per minute, which determines the length of time the feed material is in contact with the drum before drying and being scraped off by the doctor knife. Other variables are, the thickness of the feed material which is controlled by the distance between the feed rolls and the drum and the method of feeding, and also the condition of the feed material which includes concentration, physical characteristics and temperature of the feed material at the time of drying (Van Arsdel *et al*, 1973; William and Gardner, 1976; Cham, 1981; Hall and Upadhaya, 1986; Moore, 1987; Brennan, 1994).

The mash which contains the additives is normally diluted to about 80% moisture content and fed onto a drum drier. The temperature of the drum is controlled by the

steam pressure. Too high temperature cause over drying of the inner sheet surface in contact with the drum, causing the sheet to fall off before drying is complete. The flakes produced end up with high moisture content (Cording *et al*, 1957; Ayernor, 1973). The drum speed controls the residence time, whereby high drum speed corresponds to shorter residence time (Van Arsdel *et al*, 1973; Ayernor, 1973; Oyanemi, 1974).

Drum drying is also applied on cereals, milk powders, baby foods, fruits like mangoes and apples. Although temperatures reached are relatively high, the drying time is relatively short (Gooding, 1972).

### ***Textural Characteristics and Their Measurement (Instrumental and Sensory)***

The texture of the food is one of the most important quality attributes that determines its acceptability, especially of those foods which are derived from yam and cassava (Onayemi, 1985). It is manifested by a combination of physical properties perceived by the senses of touch (including kinaesthetic and mouthfeel), sight and hearing (Szczesniak, 1963; Brennan *et al*, 1970; Anon, 1975). Textural characteristics are defined and classified into mechanical (which are manifested by the reaction of food to stress) and geometrical qualities (which refer to arrangement of constituents of food, reflected mainly in appearance of food) as well as those related to moisture and fat content of the product (Szczesniak, 1963; Brennan *et al*, 1970; Brennan, 1980; Szczesniak, 1983; Giese, 1995).

Textural characteristics can be studied by: mechanical, sensory, histological, chemical and analytical methods. The mechanical methods involve instrumental measurements in

which different devices are used such as, penetrometers, compressimeters, shearing devices, consistometers, viscometers and extrusion devices. The General Foods Texturometer is an instrument which gives a combination of measurements obtained from different tests. This method is known as texture profiling, by which force curves recorded during the test can be analysed in terms of several parameters (Brennan, 1980; Bourne, 1982; Pons and Fiszman, 1996; Carballo *et al*, 1996).

The textural parameters can be defined as:

Hardness- the force necessary to attain a given deformation.

Cohesiveness- The strength of internal bonds making up the body of the product.

Viscosity- The rate of flow per unit force.

Springiness- The rate at which a deformed material goes back to its undeformed condition after the deforming force is received.

Adhesiveness (stickiness)- the work necessary to overcome the attractive forces between the surface of the other material with which the food comes in contact (Bourne, 1966; Sherman, 1969; Prentice, 1984; Giese, 1995; Pons and Fiszman, 1996).

Many investigations revealed that measurements from mechanical devices and taste panels ratings yielded statistically significant correlations (Szczesniak and Torgenson, 1965; Brennan, 1980).

Sensory methods involve identification and quantification of textural characteristics of food sample by a human assessor. The quality characteristics of foods are evaluated by sensory panels (Abbot, 1972). The five senses or receptors that are used in the perception of food are, sight, touch, hearing, smell and taste (Daget 1977). The eyes

(sight) are involved in recognition of the state, shape, colour, texture and size of the food. The sense of touch supplies information about texture, temperature, form, weight and consistency of food. Perception is possible by using hands and mouth. Hearing is associated with eating. Biting and chewing makes sounds, which are perceived and give the indication of the texture of food, and are also part of the pleasure of eating. The sense of smell is stimulated by chemical energy. Complex mixtures of odourant molecules produce smells which are perceived by the sense of smell and evoke the pleasure of eating. The taste of food is perceived by the tongue, but the character of food depends on aroma than taste alone, and the two constitute the flavour. The four fundamental tastes are salt, sweet, sour and bitter which together with other senses they form the basis of flavour in the mouth (Daget, 1977; Lawless and Claasen, 1993).

The sensory panel consists of a few people, trained to hundreds untrained randomly selected consumers (Abbot, 1972). The kind of people selected for a panel depends on the question to be answered by the panel. According to Daget (1977) the panel can be used for:

- quality control of raw materials and the end products;
- assessing the effect on the end products, of changes in either raw materials or the technology of preparation;
- monitoring the effect of packaging on the end products;
- determining the shelf-life;
- monitoring the development stages of a new product.

Different tests used by panellists are:

Preference/ Acceptance tests. These are used to evaluate the opinion of like and dislike of the consuming public, and are to be used by consumers. The tests determine which of

the two or three samples most people like best. Acceptance tests show if a product is likely to be accepted by most potential consumers. What they actually measure is the opinion of the consumers. The consumer tests, consider all attributes, not only texture. They are supposed to be conducted by panellists who accurately represent the population for which the product is intended. Whenever possible, a large size of panel is necessary, although in reality, small panels are used in the laboratory, due to costs involved.

Discriminatory tests. These are used to determine whether there are detectable differences among samples. They do not indicate magnitude or extent of difference in samples. They can be divided into threshold tests and difference tests.

The threshold test measures the intensity of a given parameter, when it can just be perceived by a given individual. It is mainly used in flavour and odour, to evaluate the level of a specific additive. The difference tests determine the difference which exists between the samples, but not its magnitude. The tests require less panellists than preference/acceptance tests, only that they need to have good sensitivity. Panellists need to be trained to recognise certain features in a set of reference standards.

Descriptive tests. They are used to show the kind or magnitude of differences between samples. These are the most preferred tests since in most studies people want to know the effect of the studied variable on food (Larmond, 1970; Abott, 1972; Kramer and Szczesniak, 1973).

The types of descriptive tests are: ranking, rating or scoring and profiling.

Ranking. In this type of test, a panellist is given several samples and asked to arrange them in order of increasing intensity of a particular attribute. This needs less training, and it shows the direction of difference.

Rating or Scoring. Here the relative intensity of a property is indicated by a mark on a rating scale or by a numerical score. Rating scales may be structured or unstructured. This type of tests require a more highly trained panel.

Another useful technique is sensory profiling which uses carefully selected and trained subjects (but no experts) to systematically translate the discernible sensory characteristics (perceptions) of a specified type of food into words and numbers. It is a technique for indirect qualitative and quantitative specification of perceptions. Scales are used to quantify the magnitude of each perception (Brandt *et al*, 1963; Abbot, 1972; Powers, 1984; Brennan, 1988; Kilcast and Lewis, 1990; Claasen and Lawless, 1992).

It is always important to match test objectives with the proper methods, selecting appropriate panellists and have correct statistical designs in order to enhance the usefulness of sensory data (Lawless and Claasen, 1993).

## Chapter 3

# DETERMINATION OF RESIDUAL CYANOGENS AND AFLATOXINS IN CASSAVA FLOUR SAMPLES FROM TANZANIAN VILLAGES

### *Introduction*

Tanzania is on the east coast of Africa. It lies between longitudes 29°30'E and 40°50'E and latitude 1°S and 11°50'S. Being in the tropics, its climate is suitable for cassava cultivation (Msabaha *et al*, 1986).

Tanzania ranks third in major cassava producing countries in Africa, after Zaire and Nigeria (FAO, 1983) (

Table 3-1). Cassava is a major source of energy for many Tanzanians, particularly in the rural population. In urban centres rice and maize are preferred to cassava. Apart from roots, cassava leaves are also consumed as green vegetables and provide a good protein source (Msabaha *et al*, 1986).

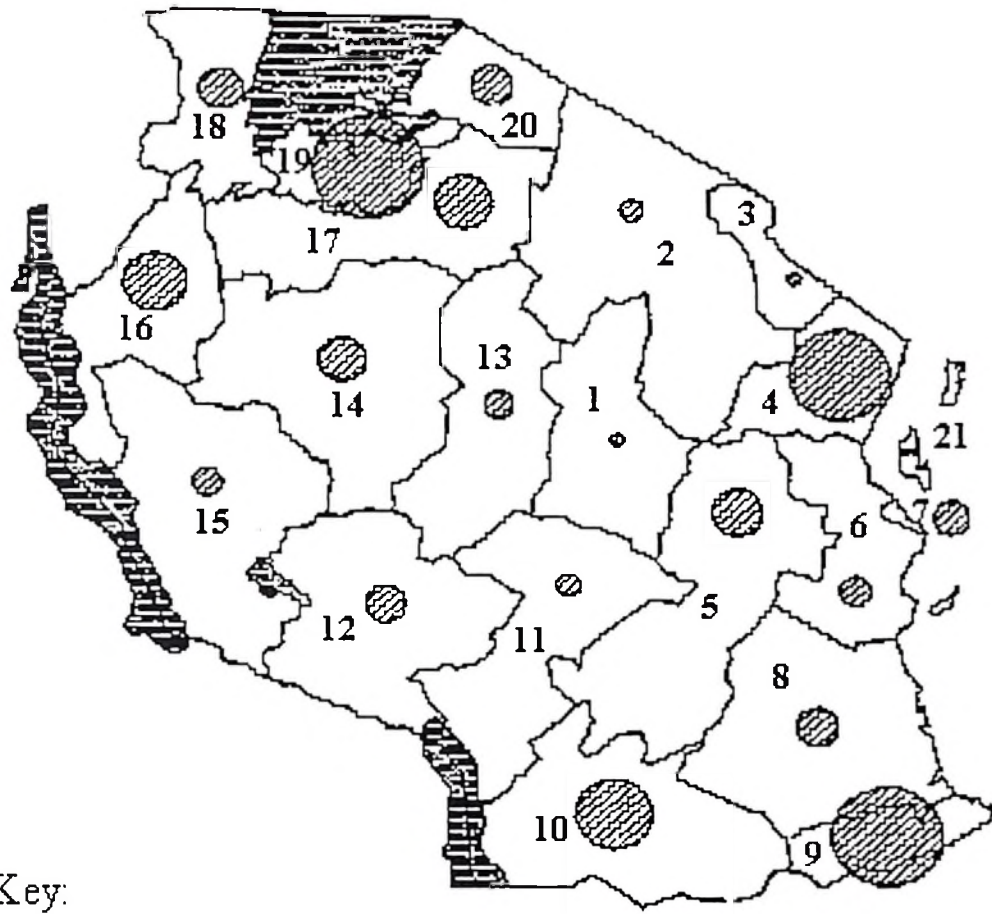
Cassava is widely grown all over the country in Tanzania. The leading regions are Mwanza, Coast, Mtwara, Tanga, Shinyanga, Zanzibar, Ruvuma and Kigoma (Figure 3-1). It is among major staple foods including maize, rice, sorghum and millet (Begg and Khamis, 1986; Nweke, 1989).

Varieties of 'bitter' and 'sweet' cassava are produced. Esser *et al* (1992) reported that bitter cassava varieties have become a staple crop in many drought-prone areas of East


**Table 3-1 Major Cassava Producing Countries in Africa**

Country	Production (Million tons)	Population (Million)	Ton/cap.
Zaire	13.6	31.2	0.42
Angola	2.00	8.30	0.24
Tanzania	4.90	21.0	0.23
Mozambique	2.90	13.0	0.22
Madagascar	1.80	9.50	0.19
Ghana	1.90	12.6	0.15
Nigeria	11.5	89.0	0.13
Uganda	1.40	14.6	0.10

Source: FAO, (1983).



Key:

 Cassava producing areas

Regions

01 Dodoma	08 Lindi	15 Rukwa
02 Arusha	09 Mtwara	16 Kigoma
03 Kilimanjaro	10 Ruvuma	17 Shinyanga
04 Tanga	11 Iringa	18 Kagera
05 Morogoro	12 Mbeya	19 Mwanza
06 Pwani	13 Singida	20 Mara
07 Dar-Es -Salaam	14 Tabora	21 Zanzibar

**Figure 3-1 Cassava Production in Tanzania**

Source: Msabaha *et al* (1986).

1 sqcm = 100,000 tons dried cassava  
Cassava production for year 1979/1980

Africa, probably because they yield better than other staples under adverse environmental conditions.

A study in Tanzania revealed that, an average of 75% of total cassava produced is processed, and only 25% is used in fresh form. Although the big proportion of processed cassava was the bitter varieties, it was found that the proportion of sweet cassava varieties processed was still substantial (Berry, 1993). This is because the consumption of fresh cassava is limited by the extreme perishability of the roots. Also with no facilities for storage and reliable transport, it is difficult to market fresh cassava roots at any distance from the place where it is grown (Berry, 1993; Nweke *et al*, 1996).

### **Cassava Processing**

Cassava processing is important for marketing of cassava as it reduces the bulk and extend shelf life (Cock, 1985; Nweke *et al*, 1996). It was also reported that fresh cassava roots have low value per unit weight, so processing adds value and improves the quality of the product which in turn expands the market by attracting higher income urban consumers (Nweke, 1992; Berry, 1993).

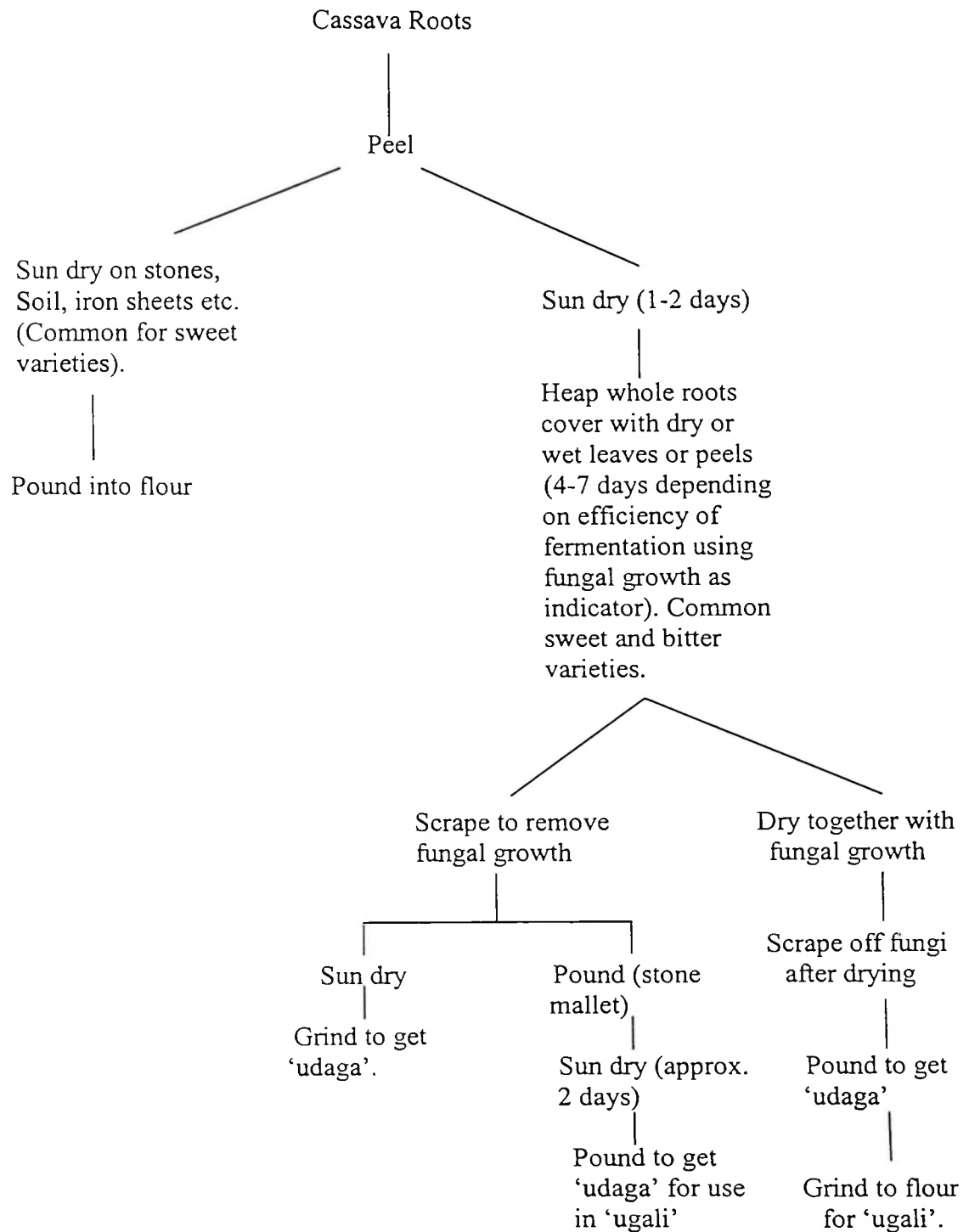
Phillips (1983) and Nweke *et al* (1994) reported that the market for certain cassava products is largely limited to low income groups, while some other forms have a significant market among high income groups. It is therefore important to improve the quality of cassava products in order to make them attractive to middle and high income earners without significant increase in processing costs. But the most important reason

for processing cassava is to remove the cyanogens (Sunderesan *et al*, 1987; Mlingi *et al*, 1995).

Traditionally cassava roots are processed by a variety of methods into many different products, depending on local customs and preferences. Cassava processing is mostly done by women and the processing methods differ depending on the end product (Nweke *et al*, 1989). The main product produced in Tanzania is cassava chips/flour. Flour is often made at home by the final consumer from dried cassava chips. So chips is an important intermediate product and constitutes a major proportion of cassava market (Berry, 1993). This is known by different names all over the country, e.g. chinyanya, ugali, makopa, obutege, omudyenge, udaga, mbute, makonga, obusanobwe or kivunde.

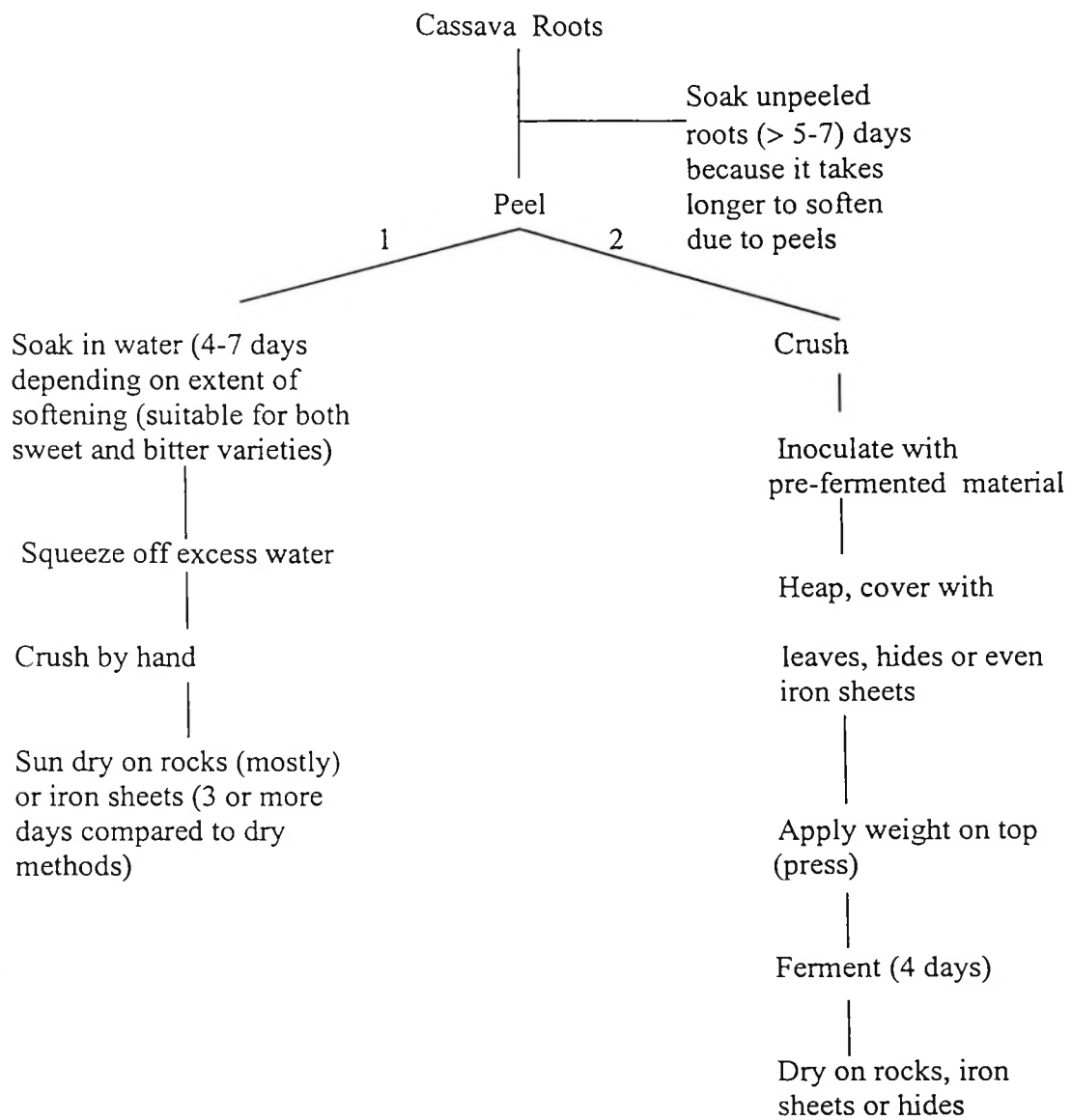
Hahn (1989) and Nweke (1994) explained that chips/flour can be made by a wide range of alternative traditional methods. These include sun drying or smoke drying of peeled fresh cassava roots. This method is mainly suitable for varieties with low cyanogenic glucoside levels. Another method is fermentation which is done by heaping or soaking the roots. This method precedes sun drying and smoke drying. The chips are then milled into flour. Figure 3-2 and Figure 3-3 show the most common processing methods processing cassava into dry chips and flour.

The flour is used to prepare ugali, but in some areas like Ngara (Kagera region) it is also used to prepare 'local' bread. The flour is wetted, made into balls, (mbute) and sun dried. Then it is cooked and eaten with sauce. In Zanzibar a similar product is called 'vipopoo'.



**Figure 3-2 Dry method of processing cassava roots**

Source: Msabaha *et al* (1986).



**Figure 3-3 Wet method of processing cassava roots**

Source: Msabaha *et al* (1986).

Mlingi *et al* (1992) reported an extensive outbreak of acute intoxications with nausea and vomiting in drought stricken district in southern Tanzania (Masasi) in 1988. Their investigations showed that the people affected were consuming insufficiently processed cassava roots. They also reported that some people in that area suffered from 'Konzo', a paralytic disease which is an epidemic upper motor neurone disease. It is a form of tropical myelopathy characterised by abrupt onset of spastic paraparesis, which causes partial paralysis, especially of lower extremities, accompanied by spasms. This disease was also found in some villages in northern Tanzania (Tarime district).

Mlingi *et al* (1991), Mlingi *et al* (1992) and Mlingi *et al* (1993) discovered that food shortage caused shortening of the established cassava processing methods. In southern Tanzania where they normally practise sun drying for about 7 days to remove the cyanogenic glucosides, in 1988 they were repeatedly pounding and drying cassava roots to obtain flour for consumption in one day. This flour did not dry completely and was found to contain high residual levels of cyanohydrins, an intermediate breakdown product of the cyanogenic glucosides.

In northern Tanzanian villages where the usual practise is fermentation (4-7 days), they were using short cuts in processing and fermenting cassava roots for overnight only. Mlingi *et al* (1995) reported that the short cut processing methods were triggered by food shortage due to drought and crop failure. Thiocyanate (SCN) which is the detoxification product of cyanogenic glucosides was used as an indicator in determining cyanide exposure. It was measured in serum and urine.

Processing methods such as sun drying and solid-state fermentation, encompass a range of conditions under which mould growth is likely to occur (Clerk and Caurie, 1968;

Mota and Lourenco, 1974; Esser and Nout, 1989; Westby *et al*, 1995). Moulds have been reported to produce mycotoxins, the common type found in cassava being aflatoxins (Clerk and Caurie, 1968; Moreau, 1979). Wareing and Westby (1993) explained that if cassava flour or chips are inadequately dried and then exposed to high humidities or in some way rewetted during storage, they will be susceptible to mould growth. The moisture content of the stored products is an important determining factor for mould growth.

According to a study carried out in Mwanza region by Thomson *et al* (1996) cassava sometimes has mould growth of different colours including grey, yellow and red. The grey mould growth occurs more often and gives the product a darker overall colour. While some consumers prefer white cassava flour, and scrape off moulds before grinding/milling the chips, some prefer dark coloured flour and do not scrape off mould.

#### ***Cassava Samples Collection and analysis***

Cassava samples analysed in this study were collected from three regions: Tanga, Mwanza, and Kagera depending on the type of processing practised and samples were taken from three villages for each processing method (Figure 3-4).

Tanga region is on the east coast of Tanzania, along the Indian ocean. In this region both wet and solid-state fermentation are practised. Solid-state fermentation is practised by Digo in Tanga district, while wet fermentation by the Bondei in Muheza district. The solid-state fermented cassava chips are milled into flour without prior scraping off the mould.



Key:

1. Mwanza region
2. Kagera region
3. Tanga region

**Figure 3-4 Map of Tanzania showing areas where cassava samples were collected from**

Source: Wright *et al* (1997).

In Tanga, solid-state fermented samples were collected from Pande, Doga and Mwakidili villages, while wet fermented samples were collected from Mkuzi, Mianga and Kihulu villages. Wet fermentation used is as shown in Figure 3-3 route 1.

Ukerewe is a very large island in Lake Victoria. It is in the Mwanza region. The type of cassava processing here is also solid-state fermentation but the mould is scraped off before milling cassava chips into flour. Samples were collected from three villages named, Kazilankanda, Mukasika and Namagondo.

Another region where cassava samples were collected from is Kagera. This is in the north west part of Tanzania, and around Lake Victoria. The type of processing practised is sun drying of thin slices and in many cases they dry sweet cassava varieties. The villages where samples were collected from are, Ibaraizibu, Kakoni and Ruzinga.

### ***Materials and Methods***

Six samples of cassava chips were collected per village from three villages in each region. Six households were chosen at random in each village. All the samples were collected from the home use stock, not for selling.

Samples were pounded into flour, packed into polythene bags and transported to Britain where they were kept in a cold room (-18°C) and analysed for residual cyanogens and aflatoxins.

## **Cyanogens determination**

Region	sample	villages	Total samples
Tanga - Solid-state fermentation	6	3	18
Tanga - Wet fermentation	6	3	18
Mwanza - Solid-state fermentation	6	3	18
Kagera - Sun drying	6	3	18

A total of 72 cassava samples of 1 kg each were collected. The samples were then pounded in a 'Kinu' (local wooden motor and pestle) and sieved to obtain fine flour. The flour was mixed thoroughly and 200 g were sub-sampled from each sample for total, intermediate and free cyanide analysis according to O'Brien *et al* (1991) method. Details of the methodology are in Appendix 2.

## **Moisture Determination**

The moisture content was determined by using a thermostat vacuum oven (Townson & Mercer Ltd. Croydon). About 2 g of cassava flour were dried at 70°C and vacuum 84.65 kNm<sup>-2</sup> for 12 hours.

## ***Aflatoxins Detection***

Aflatoxins were analysed in samples which were solid-state fermented and sun dried only. Cassava samples (54) of 2 kg each were collected, pounded in a 'Kinu' and sieved into fine flour. After a thoroughly mixing, 200 g were sub sampled from each sample,

and screened for aflatoxins. The method used for aflatoxins determination was according to Park *et al* (1994) and AOAC (1990), details in Appendix 2.

### ***Results and Discussion.***

The cyanogens levels in cassava flour processed by solid-state fermentation in Tanga and Mwanza regions are shown in Table 3-2 and Table 3-3. The average total cyanogens for cassava samples from Tanga region villages is 13.89 mg HCN.kg<sup>-1</sup> and the average total cyanogens for cassava samples from Mwanza region is 13.95 mg HCN.kg<sup>-1</sup> (Figure 3-5). These were not significantly different ( $p \geq 0.05$ ). Again there is no significant difference in total cyanogens levels of cassava samples in villages within regions ( $p \geq 0.05$ ) (Appendix 3).

In the two regions, solid-state fermentation is carried out in the same way, only that in Mwanza the mould is scraped off before milling cassava into flour, while in Tanga no scraping off of mould is done. The total cyanogens content for both regions is slightly above the safe level recommended by FAO of 10 mg HCN.kg<sup>-1</sup>.

In solid-state fermentation mould growth is said to contribute to cyanogens loss by means of enzymatically induced cellular disruption and by production of  $\beta$ -glucosidases (Esser and Nout, 1989; Gidamis *et al*, 1993). Also linamarase- type activity has been observed in certain yeast and moulds (Okafor and Ejiofor, 1986; Padmaja and Balagopal, 1985; Muzondo and Zvauya, 1995).

## Residual cyanogens in cassava flour samples from Tanga region

*Tanga : Solid-state fermentation, no scraping off moulds*

**Table 3-2 Cyanogens\* levels in solid-state fermented cassava flour samples from different villages in Tanga (mg HCN /kg )**

	Village 1	Village 2	Village 3
Total cyanogens	14.4 (2.13)	13.7 (2.24)	13.4 (1.16)
Intermediate cyanide	0.55 (0.32)	0.33 (0.10)	0.35 (0.16)
Free cyanide	1.08 (0.25)	0.98 (0.22)	0.82 (0.21)
Cyanogenic glucosides	12.8 (1.83)	12.4 (2.27)	12.2 (1.10)

\* Cyanogens values are an average of six samples. Standard deviation values are in brackets.

Variables used to calculate cyanogens content are shown in Appendix 1. All results given for cyanogens are presented on dry weight basis.

## Residual cyanogens in cassava flour samples from Mwanza region

*Mwanza : Solid-state fermentation, moulds scraped off*

**Table 3-3 Cyanogens\* levels in solid-state fermented cassava flour samples from different villages in Mwanza (mg HCN /kg )**

	Village 1	Village 2	Village 3
Total cyanogens	14.3 (2.09)	14.5 (2.07)	13.1 (1.17)
Intermediate cyanide	0.41 (0.17)	0.30 (0.09)	0.23 (0.12)
Free cyanide	0.87 (0.25)	0.89 (0.27)	0.78 (0.26)
Cyanogenic glucosides	13.0 (2.08)	12.5 (1.35)	12.1 (1.16)

\* Cyanogens values are an average of six samples. Standard deviation values are in brackets.

Gidamis *et al* (1993) reported 58% reduction in total cyanogens in air fermented (solid-state fermented) roots, in which subsequent drying further reduced the total cyanogens to about 9% of the original content.

In this study, although the original cyanogens content of cassava samples is unknown, it can be confidently assumed that there was reduction of the cyanogens during fermentation, since cassava samples were from the bitter cassava varieties. Bitter cassava varieties have cyanogens content of above 100 mg HCN.kg<sup>-1</sup> (Nartey, 1978). Again we cannot compare directly the processing methods in this study because they were performed in different places by different people and on different samples. But the trend observed in this case is similar to that observed by Gidamis *et al* (1993) who processed cassava roots and found that wet fermentation causes higher losses in total cyanogens than solid-state fermentation and sun drying.

Mahungu *et al* (1987) also observed that solid-state fermentation is not as effective as that in water. But studies have shown that solid-state fermentation is more effective than sun drying alone (Esser *et al*, 1992; Gidamis *et al*, 1993).

Flour from cassava samples processed by solid-state fermentation in both Tanga and Mwanza regions had low cyanohydrin content (Table 3-2, Table 3-3 and Figure 3-6). This may be due to gradual hydrolysis of cyanogenic glucosides to cyanohydrins and free cyanide, due to slow enzyme penetration. Also high pH would permit the rapid hydrolysis of cyanohydrins (Gidamis *et al*, 1993).

The residual cyanogens are mainly cyanogenic glucosides (bound cyanogens). This is given by total cyanogens values minus intermediate and free cyanide. Free cyanide

levels were low (Figure 3-7), this is because they can easily evaporate at 28°C (Fomunyan *et al*, 1985).

Table 3-4 shows the cyanogens levels in cassava flour samples from Tanga region, which were processed by wet fermentation. The average total cyanogens content was 5.84 mg HCN.kg<sup>-1</sup>. This is within the recommended safe levels of less than 10 mg HCN. kg<sup>-1</sup>. Residual total cyanogens in cassava samples from different villages were not significantly different ( $p \geq 0.05$ ). Wet fermentation is said to facilitate breakdown of cyanogenic glucosides to low total cyanogens levels (Mahungu *et al*, 1977; Maduagwu, 1979; Vasconcelos *et al*, 1990; Muzanila, 1993; Agbor-Egbe *et al*, 1995). It is capable of an overall reduction of up to 99% (Padmaja 1995).

Agbor-Egbe *et al* (1995) reported that despite the wide range in cyanogens glucoside content of freshly harvested cassava roots, they observed similar reduction levels in the fermented product.

Dipping of cassava roots in water makes them soft and causes the cells to rupture, permitting linamarase to act on cyanogenic glucosides (Padmaja *et al*, 1982; Okafor *et al*, 1984; Ayernor, 1985; Okolie and Ugochwuku, 1988; Oyewole and Odunfa, 1992). Lactic acid bacteria play an important role in cassava wet fermentation (Okafor, 1978; Abe and Lindsay, 1978; Ngaba and Lee, 1979; Oyewole and Odunfa, 1988). *Lactobacillus plantarum* was found to be predominant and produce a linamarase enzyme, with similar physiological properties to those produced by endogenous cassava plant materials (Ikediobi and Onyike, 1982a; Agbor-Egbe *et al*, 1995). Oyewole and Odunfa (1991) said that detoxification during submerged fermentation where roots are

*Tanga : Wet fermentation*

**Table 3-4. Cyanogens\* levels in wet fermented cassava flour samples from different villages in Tanga (mg HCN /kg )**

	Village 1	Village 2	Village 3
Total cyanogens	5.51 (1.75)	5.62 (3.53)	6.43 (2.85)
Intermediate cyanide	4.38 (1.71)	4.57 (3.20)	5.37 (2.66)
Free cyanide	1.13 (0.58)	1.15 (0.63)	0.97 (0.37)
Cyanogenic glucosides	-0.22 (0.57)	-0.05 (0.50)	0.12 (0.63)

\* Cyanogens values are an average of six samples. Standard deviation values are in brackets.

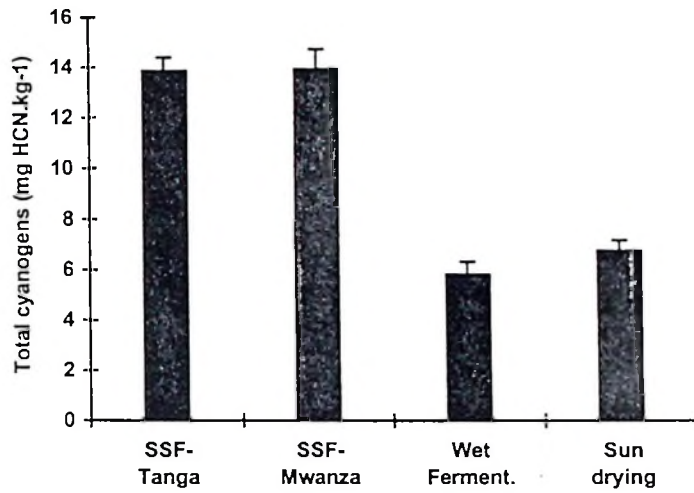
**Residual cyanogens in cassava flour samples from Kagera region.**

*Kagera: Sun drying.*

**Table 3-5 Cyanogens\* levels in sun dried cassava flour samples from different villages in Kagera (mg HCN /kg )**

	Village 1	Village 2	Village 3
Total cyanogens	7.10 (3.38)	6.35 (1.13)	6.95 (2.53)
Intermediate cyanide	1.03 (0.58)	0.32 (0.15)	0.27 (0.12)
Free cyanide	0.79 (0.26)	0.88 (0.15)	0.67 (0.15)
Cyanogenic glucosides	4.30 (2.06)	5.13 (1.26)	6.02 (2.57)

\* Cyanogens values are an average of six samples. Standard deviation values are in brackets.



**Figure 3-5 Total cyanogens in cassava flour samples processed by different methods**

Mean results from 3 villages within each region (18 samples)

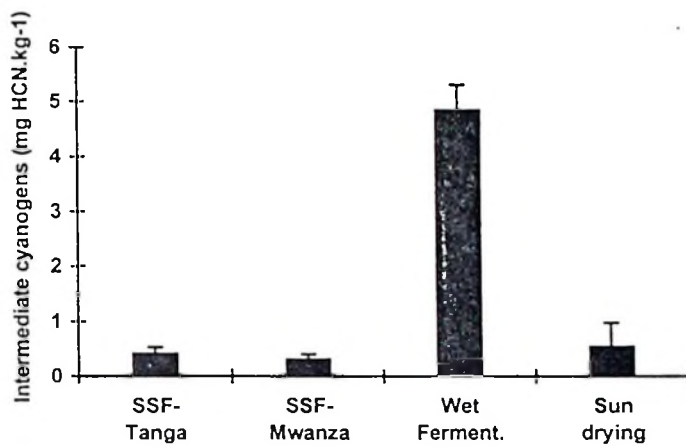
Error bars derived from S.D. values

SSF- Tanga - Solid State Fermentation in Tanga region

SSF-Mwanza- Solid State Fermentation in Mwanza region

Wet-Fermentation - Wet Fermentation in Tanga region

Sun drying - Sun drying in Kagera region



**Figure 3-6 Intermediate cyanide (Cyanohydrins) in cassava flour samples processed by different methods**

Mean results from 3 villages within each region (18 samples)

Error bars derived from S.D. values

SSF- Tanga - Solid State Fermentation in Tanga region

SSF-Mwanza- Solid State Fermentation in Mwanza region

Wet-Fermentation - Wet Fermentation in Tanga region

Sun drying - Sun drying in Kagera region

not grated, involve enzymes from both plant material and micro-organisms. The Residual cyanogens in cassava flour samples from Tanga region optimum linamarase activity was obtained at pH 5.7 with a temperature range of 30-40°C.

It has also been reported that, cyanogenic glucosides removal can be enhanced by direct leaching into the soaking water (Nambisan and Sundaresan, 1985; Hahn, 1989; Vasconcelos *et al*, 1990; Sokari and Karibo, 1992).

Table 3-4 and Figure 3-6 show that the main residual cyanogens in the wet fermented cassava flour is cyanohydrins. The cyanogenic glucosides are very low. This is because soaking and fermentation facilitate contact between endogenous enzyme linamarase and the cyanogenic glucosides substrate. Lactic acid bacteria cause pH reduction during fermentation (Okafor *et al*, 1984; Nwachukwu and Edwards, 1987; Muzanila, 1993). If

the system becomes acidic a greater proportion of cyanohydrins is retained in the cassava as low pH inhibits cyanohydrins breakdown (Vasconceles *et al*, 1990; O'Brien *et al*, 1992; Agbor-Egbe *et al*, 1995).

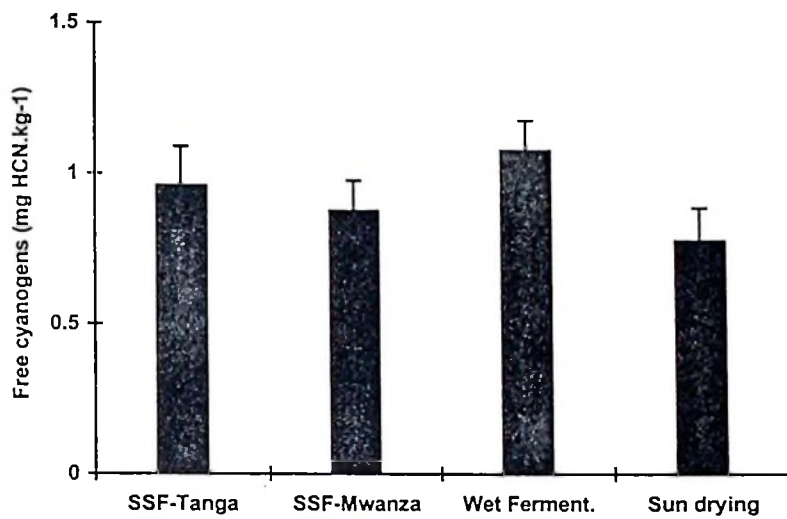
Table 3-5 shows the cyanogens levels of cassava flour samples processed by sun drying in Kagera region. The mean of total cyanogens was 6.8 mg HCN.kg<sup>-1</sup>. According to Appendix 3, samples from different villages did not show significant difference in residual total cyanogen ( $p \geq 0.05$ ). Although this is within the safe levels of 10 mg HCN.kg<sup>-1</sup> as recommended by FAO, it should be remembered that all samples collected in this region were from sweet cassava varieties. The villagers said that they use sun drying for sweet varieties only, and in the case of bitter varieties they soak them in water.

Therefore, the residual total cyanogens in cassava samples was low probably because the samples did not have high cyanogenic glucosides content to start with.

Substantial reductions in cyanogenic glucosides have been reported in cassava drying experiments. According to Gomez and Valdiviessa (1984) total cyanogen losses of up to 80% can be achieved during oven drying of cassava chips and up to 94% during sun drying. Chipping or slicing allows contact between linamarase enzyme and cyanogenic glucosides. Sun drying is reported to be more effective in cyanogenic glucosides detoxification than oven drying, because the longer drying period allows more contact time between linamarase and cyanogenic glucosides.

Mlingi *et al* (1995) reported that sun drying of whole cassava roots does not achieve sufficient cell disintegration to bring the linamarase enzyme into contact with cyanogenic glucosides to achieve hydrolysis. But on the other hand, thin slices can dry very fast, thus reducing the moisture content to a level that inactivates the enzyme before tissue disruption enabled it to act on the cyanogenic glucosides. Although sun drying of bitter cassava varieties reduces the amount of cyanogenic glucosides considerably, about 10% of the initial cyanogenic glucosides remain in flour (Anon, 1984; Mlingi *et al*, 1992; Mlingi *et al*, 1995). Cassava peel contains higher enzyme concentration so the rate of cyanogenic glucosides hydrolysis is higher in unpeeled roots.

The majority of residual cyanogens in the sun dried cassava samples in Kagera region was in form of cyanogenic glucosides (Table 3-5 and Figure 3-8). The possible explanation is that the remaining cyanogenic glucosides were never exposed to the enzyme until moisture content became too low for enzymatic hydrolysis. Cyanohydrins



**Figure 3-7 Free cyanide (HCN) in cassava flour samples processed by different methods**

Mean results from 3 villages within each region (18 samples)

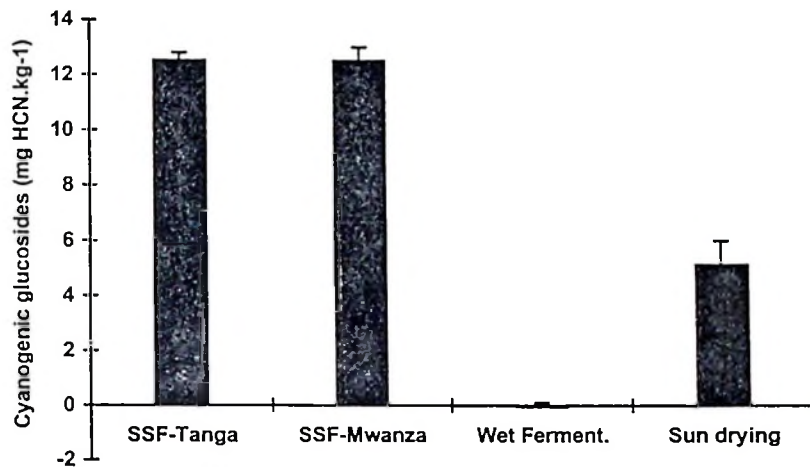
Error bars derived from S.D. values

SSF- Tanga - Solid State Fermentation in Tanga region

SSF-Mwanza- Solid State Fermentation in Mwanza region

Wet-Fermentation - Wet Fermentation in Tanga region

Sun drying - Sun drying in Kagera region



**Figure 3-8 Cyanogenic glucosides (Bound cyanogens) in cassava flour samples processed by different methods**

Mean results from 3 villages within each region (18 samples)

Error bars derived from S.D. values

SSF- Tanga - Solid State Fermentation in Tanga region

SSF-Mwanza- Solid State Fermentation in Mwanza region

Wet-Fermentation - Wet Fermentation in Tanga region

Sun drying - Sun drying in Kagera region

levels were low as shown in Table 3-5 and Figure 3-6. Banea *et al* (1992) explained that in sufficiently dried flour, very low cyanohydrin and free cyanide are found. They further said that, while completeness of disintegration before drying determines cyanogenic glucoside removal, cyanohydrins removal depends only on completeness of subsequent drying. In all cases free cyanide is rapidly lost and does not normally contribute to dietary cyanogens (Mlingi *et al*, 1995). This is also shown in Figure 3-7.

Figure 3-9 shows that most of cassava samples processed by wet fermentation, had residual total cyanogens levels below 10 mg HCN.kg<sup>-1</sup>. The probability of samples containing residual total cyanogens levels greater than 10 mg HCN.kg<sup>-1</sup> is 6.1% (Appendix 3). Likewise, most of sun dried cassava samples from Kagera region processed by sun drying had residual total cyanogens levels below 10 mg HCN.kg<sup>-1</sup>. Appendix 3 show that the probability of these samples containing residual total cyanogens levels greater than 10 mg HCN.kg<sup>-1</sup> is 9%.

But most of samples processed by solid-state fermentation from Tanga and Mwanza regions had residual total cyanogens levels above 10 mg HCN.kg<sup>-1</sup>, (Table 3-2 and Table 3-3) the probability of these samples containing residual total cyanogens lower than 10 mg HCN.kg<sup>-1</sup> being 2.2% and 1.9% respectively (Appendix 3).

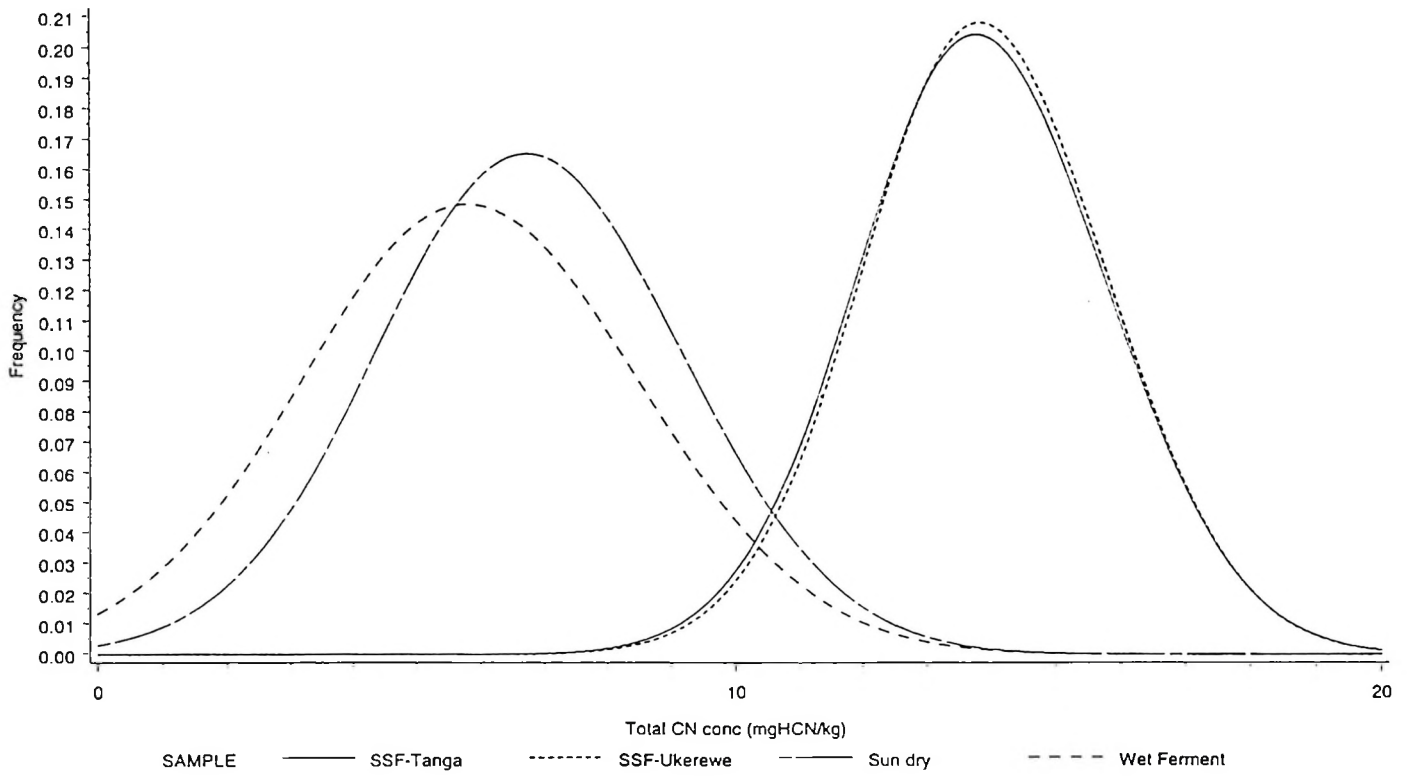


Figure 3-9 Normal distribution curves for residual total cyanogens levels in cassava samples

## ***Conclusions***

Cassava samples processed by wet fermentation comply to the safe level of not more than 10 mg HCN.kg<sup>-1</sup>. The same applies to samples processed by sun drying. samples processed by solid-state fermentation exceeded the safe level, it is by about 4mg HCN.kg<sup>-1</sup>. Again the residual cyanogens are in the form of cyanogenic glucosides (bound cyanogens). Barret *et al* (1977) and Mlingi *et al* (1995) reported that animal experiments indicated that ingested linamarin can be absorbed unchanged and excreted intact in urine. They further said that ingestion of linamarin can result in cyanide exposure if suitable microbial glucosidases are present in the gut and this mechanism is not confirmed as occurring in humans.

Mlingi *et al* (1992) conducted a study in Masasi district in southern Tanzania and found that people who consume flour with high cyanogenic glucosides levels, had low urinary thiocyanate levels, indicating that the cyanogenic glucosides have very low cyanide exposure. It has been reported that cyanide exposure may result from cyanohydrins. This is the main residual cyanogen in wet fermented flour. Ingested cyanohydrins will yield hydrogen cyanide in the alkaline environment in the gut and may be much more toxic than equimolar amounts of cyanogenic glucosides (Mlingi *et al*, 1995).

However, cyanohydrins decompose on storage at ambient temperature and fall to negligible levels (Mahungu *et al*, 1987; O'Brien *et al*, 1992). Mlingi *et al* (1995) suggested that most of the processing methods practised in Tanzania are not very effective in reducing cyanogens in roots from bitter cassava varieties with high cyanogenic glucoside levels. They argued that wet fermentation which has been found to be effective but cannot be practised in some of the areas, due to water shortage.

They further said that, in northern Tanzania where peeled cassava roots were crushed with a wooden mallet before heap (solid-state) fermentation, the cyanogenic glucosides were reduced to low levels. This is because, hammering causes further cell disintegration. This can be compared to grating, which is applied in West Africa. Grating of cassava roots followed by sufficient sun drying reduce cyanogens to negligible levels (Nambisan and Sundaresan, 1985; Hahn, 1989; Vasconcelos *et al*, 1990). This is the principle used in gari processing in West Africa.

This study indicates that, cassava processed by wet fermentation in Tanzanian villages is safe. From this study it can be recommended that cassava eating population who cannot practise wet fermentation because of their environment or climatic conditions, need to be advised, encouraged and trained to practice grating followed by solid-state fermentation. This will reduce the cyanogens content to safe levels.

This can take some time, because it means teaching them new skills. Grating itself is laborious but where possible (where resources can allow) some mechanical graters can be used. In West Africa mechanical graters are widely used in villages.

Most of the work about cassava detoxification has been done in the laboratory, also some studies were conducted during the food shortage crisis, which revealed high levels of residual cyanogens in processed cassava. But this study has revealed that, under normal conditions, when there is no famine, cassava products processed in the villages, by wet fermentation are safe to eat.

### ***Aflatoxins Results.***

Cassava samples processed by solid-state fermentation and sun drying contained no aflatoxins. Cassava samples were screened for aflatoxins. All samples did not show the blue fluorescence under the UV light. In that case tests for quantification of aflatoxins were not carried out. Aflatoxin B<sub>1</sub> standards were used since this is the most abundant and likely to be produced in cassava. Studies showed that *Aspergillus* species produce aflatoxin B<sub>1</sub> and G<sub>1</sub> at the same time in cassava. So the absence of aflatoxin B<sub>1</sub> indicates the absence of aflatoxin G<sub>1</sub> too. Westby *et al* (1995) found that *A. parasiticus* C7 produce aflatoxins B<sub>1</sub> and G<sub>1</sub> on cassava, while *A. flavus* A41, *A. parasiticus* C7 and *A. parasiticus* MO39 produced aflatoxins B<sub>1</sub> and G<sub>1</sub> in rice. They also observed that *A. flavus* A38, *A. flavus* A39 and *A. flavus* A44 produce aflatoxin B<sub>1</sub> only without G<sub>1</sub> in rice.

Wheatley (1984), Cock (1985) and Westby *et al* (1995) explained about the confusion which existed in literature, over the ability of cassava to support aflatoxin production, because of contamination of assay with scopoletin. Waering and Westby (1991) reported that there is evidence cassava can support aflatoxin production under suitable conditions. There is a risk of aflatoxin development during storage under warm and humid conditions (Tsai *et al*, 1984).

In Tanzania storage conditions of cassava may sometimes be conducive for aflatoxin production, because of high ambient temperatures (25-30°C). Cassava products are normally stored in sisal sacks or open tins and thus could pick up moisture. Masimango *et al* (1977) reported that some cassava samples in Zaire contained aflatoxin B<sub>1</sub> levels greater than 1 ppm. They observed the optimum temperature range for aflatoxins

production by *A. flavus* as 25-30°C, and RH greater than 80%. They concluded that potential carcinogenic danger exists and suggested that mould proliferation on cassava should be prevented. In Mozambique, Mota and Lourenco (1974) found that cassava stored under humid conditions had rapid mould growth and aflatoxin B<sub>1</sub> (0.5 - 4.61 ppm). In South Africa aflatoxins were prevalent in cassava and foods containing groundnuts (Rensburg *et al*, 1975).

Cassava samples in this study were not contaminated with aflatoxins, possibly because they were processed in the dry season, so dried quickly to the low moisture content, which would not allow aflatoxins production. Northolt *et al* (1977) and Martin and Lloyd (1982) reported that aflatoxins B<sub>1</sub> can be produced by *A. flavus* and *A. parasiticus* at a<sub>w</sub> above 0.83. Christensen *et al* (1973) and Mirocha *et al* (1980) reported that potentially toxin producing fungus can only produce toxin when present in pure culture, but if growing along with a mixture of other moulds, then the toxin is not produced or is metabolised.

Esser *et al* (1995) did not detect any aflatoxins in cassava flour collected in rural households in Uganda. They found the dominant mycelial growth to be *Neurospora sitophila*, *Geotrichum candidum* and *Rhizopus oryzae*. Sajise and Ilag (1987) also found that *A. flavus* and *A. parasiticus* can easily grow on cassava especially in the absence of competing moulds, but not necessarily a good substrate for aflatoxins production. In another study by Essers and Nout (1989) traditionally processed cassava segments stored for 8 months, has fungal growth but no aflatoxins were detected. In Nigeria, no aflatoxins were detected in gari samples too (Ukun and Iwenor, 1989).

Sardjono *et al* (1995) reported that production of aflatoxins by *Aspergillus flavus* in mixed culture with *A. Oryzae* was significantly reduced (15 folds). They further observed that aflatoxin B<sub>1</sub> present in the spent culture medium of *A. flavus* was deactivated by subsequent growth of *A. oryzae*. Lactic acid bacteria are also reported to inhibit the growth and production of aflatoxin by *Aspergillus* species (Gourama and Bullerman, 1995). *Lactobacillus casei* and *Lactococcus lactis* reduced quantities of aflatoxins formed by *A. parasiticus*. Also *L. cremoris* and *L. lactis* were able to metabolise the preformed aflatoxins to hydroxylated derivatives of lower toxicity (Crocini *et al*, 1995).

A study by Munimbazi (1996) in Burundi showed that *Aspergillus flavus* and *A. parasiticus* were capable of producing aflatoxins but were not able to produce aflatoxins in the presence of *Bacillus pumilus*. *Bacillus pumilus* was found to produce anti fungal compound. This extracellular metabolite inhibits mycelial growth and /or mycotoxin production of many *Aspergillus*, *Fusarium* and *Penicillium* species. The predominant moulds found in cassava were *Penicillium citrinum*, *P. corylophilum* and *P. chrysogenum*.

### **Conclusion.**

It is speculated that if samples were collected in rainy season, when it is difficult to dry cassava, the results would have been different. Since contamination by mycotoxins producing moulds is likely to occur during storage, it is important to make sure that cassava products are well dried and avoid dampness during storage. But again, the judgement of consumer safety cannot be adequately carried out by looking at the mould growth on cassava.

## Chapter 4

# CHEMICAL COMPOSITION OF CASSAVA FLOUR SAMPLES FROM TANZANIAN VILLAGES PROCESSED BY DIFFERENT METHODS

### *Introduction*

In Tanzania, Protein Energy Malnutrition (PEM) is among the nutritional problems currently being experienced (Mosha and Svanberg, 1990). This is attributed to the diet which is low in energy and protein especially in some regions where diet has traditionally been based on staple root crops of cassava.

Cassava is essentially a starchy food. Its composition shows that it has low protein, mineral and vitamin content, with the exception of vitamin C (Purseglove, 1968; Grace, 1977; Okezie; Lancaster *et al*, 1982 and Kosikowski, 1982).

Low protein staples such as cassava do not provide adequate protein for human requirements even when ingestion exceeds caloric requirements. This is in contrast to diets based on cereals (8-10% protein) which can meet adult protein requirements (Cheftel *et al*, 1985).

Cook (1982) said that a diet of cassava alone can cause chronic deficiency-related diseases in the long term. Cassava is usually eaten with soup, which depending on the family income, may or may not supplement the low protein of cassava. But although the majority of people cannot afford the expensive animal protein, they eat beans and leafy vegetables which are also relatively rich in protein.

Traditional processing of cassava has both advantageous and detrimental effects. Cassava processing including fermentation and drying reduces the vitamin and mineral content (Lancaster *et al*, 1982; Ezeala, 1984). Pyke (1979) reported the loss of vitamin C in gari processing. The vitamin C recommended daily allowance for humans, ranges between 15-60 mg depending on age, sex and physiological status (Pyke, 1979).

Fermentation has been traditionally practised in many African and Latin American countries, as a means of improving texture and imparting flavour to the product (Oyenuga, 1968; Oyewole and Odunfa, 1989; Mathew George *et al*, 1995). Wet fermentation causes losses of protein (Ezeala, 1984; Bokanga *et al*, 1988; Ayankunbi *et al*, 1991; Padmaja *et al*, 1994). Favier *et al* (1971) reported losses between 50-87% of the original protein value. Padmaja *et al* (1994) said that decrease in protein is likely to result partly from the leaching into steeping water.

Sun drying also has been reported to cause slight decrease in protein content (Maini *et al*, 1981; Fay, 1991). Again sun drying has also been reported to cause losses in vitamin C (Lingel *et al*, 1994).

Solid-state fermentation causes an increase in protein content (Zvauya and Muzondo, 1994). The technique has been adopted for protein enrichment in cassava. A number of workers have reported the use of micro-organisms to convert cassava starch into microbial protein in a solid-state fermentation process (Azoulay, 1978; Hasseltine, 1980; Raimbault *et al*, 1985; Zvauya and Muzondo, 1994; Soccol *et al*, 1994). The most common used moulds are *Aspergillus niger*, *Aspergillus oryzae*, *Candida utilis*, *Candida tropicalis*, and *Rhizopus* species.

This kind of protein enrichment is also applied to other products, like apple pomace (Bhala and Joshim, 1994). Normally an externally added nitrogen is required for the micro-organisms (Okafor, 1977; Ezeala, 1984; Antai and Mbongo, 1994).

It was not known whether the chemical composition of cassava flour processed by different methods is the same. Therefore, the purpose of this study is to analyse cassava flour processed by different methods in Tanzanian villages, and find out how each method affected the chemical composition of cassava flour.

Samples were collected from three regions, already explained in Chapter 3. The samples analysed in this study are the same as those used for cyanogens analysis in Chapter 3.

## ***Materials and Methods***

### **Starch determination**

The acid hydrolysis method according to the AOAC (1995), (method 22.) was followed with some modifications as described by Rickard and Behn (1987).

### **Vitamin C determination**

Vitamin C content was determined using the Microfluorometric method of AOAC Methods (1980).

### **Reducing sugars determination.**

The reducing sugars determination was carried out by the Lane and Eynon's Volumetric method as described by Egan *et al* (1981). The volume of Fehling's solution used for

titration was 10 ml. Sample preparation and sugar extraction was done according to AOAC Official Methods of Analysis (1995). Moisture content, protein and pH determinations were carried out according to AOAC Official Methods of Analysis (1995).

### ***Results and Discussion***

The chemical composition of cassava flour sample processed by solid-state fermentation in Tanga and Mwanza regions are shown in Table 4-1 and Table 4-2. The chemical composition of cassava flour samples from the two regions was not significantly different ( $P \leq 0.05$ ). Likewise the samples in different villages within the regions.

The chemical composition of cassava flour samples processed by wet fermentation are shown in Table 4-3. These were also collected from Tanga region.

Sun dried samples from Kagera region were also analysed for their chemical composition, which are presented in Table 4-4.

Figure 4-1 shows the average starch content of cassava flour samples processed by different methods.

In this study, the original chemical composition of cassava before processing is not known, but by comparing the results with the values given in literature, it is possible to see how these processing methods have affected the chemical composition of cassava samples.

## Chemical composition of cassava flour from Tanga region

Tanga: Solid -state fermentation, no scraping off moulds.

All results given are presented on dry weight basis.

**Table 4-1 Chemical composition\* value of cassava flour from different villages in Tanga**

	Starch	Reducing sugars	Protein	Vitamin C	Moisture	pH
	(g/100g)	(g/100g)	(g/100g)	(mg/100g)	(g/100g)	
Village	80.0	6.65	2.47	29.6	14.5	5.13
1	(1.46)	(0.63)	(0.21)	(2.33)	(0.94)	(0.12)
Village	80.3	6.05	2.58	27.4	14.3	5.25
2	(1.87)	(0.53)	(0.23)	(1.95)	(0.71)	(0.26)
Village	80.2	5.78	2.72	29.8	13.8	5.25
3	(1.86)	(0.51)	(0.25)	(3.58)	(0.72)	(0.19)

\* Values are an average of six samples. Standard deviation values are in brackets.

## Chemical composition of cassava flour from Mwanza region

Mwanza region: Solid -state fermentation, moulds scraped off.

All results given are presented on dry weight basis.

**Table 4-2 Chemical composition\* of cassava flour from different villages in Mwanza**

	Starch (g/100g)	Reducing sugars (g/100g)	Protein (g/100g)	Vitamin C (mg/100g)	Moisture (g/100g)	pH
Village 1	77.78 (1.48)	7.20 (0.19)	2.20 (0.24)	24.52 (1.46)	13.44 (0.79)	5.33 (0.22)
Village 2	78.87 (1.47)	6.85 (0.26)	2.23 (0.29)	23.57 (1.24)	13.82 (0.87)	5.25 (0.230)
Village 3	77.93 (1.29)	6.78 (0.26)	2.23 (0.26)	25.31 (1.00)	13.51 (0.88)	5.23 (0.15)

\* Values are an average of six samples. Standard deviation values are in brackets.

## Chemical composition of cassava flour from Tanga region

Tanga: Wet fermentation.

All results given are presented on dry weight basis.

**Table 4-3 Chemical composition\* of cassava flour from different villages in Tanga**

	Starch	Reducing sugars	Protein	Vitamin C	Moisture	pH
	(g/100g)	(g/100g)	(g/100g)	(mg/100g)	(g/100g)	
Village	72.6	3.03	0.75	15.4	13.7	5.25
1	(3.2)	(0.22)	(0.13)	(1.42)	(0.71)	(0.19)
Village	69.3	3.08	0.66	18.6	14.1	4.28
2	(1.29)	(0.39)	(0.09)	(2.70)	(0.77)	(0.19)
Village	72.5	2.88	0.82	17.8	13.8	4.35
3	(5.90)	(0.25)	(0.07)	(2.17)	(0.41)	(0.21)

\* Values are an average of six samples. Standard deviation values are in brackets.

## Chemical composition of cassava flour from Kagera region

Kagera: Sun drying.

All results given are presented on dry weight basis.

**Table 4-4 Chemical composition\* of cassava flour from different villages in Kagera**

	Starch (g/100g)	Reducing sugars (g/100g)	Protein (g/100g)	Vitamin C (mg/100g)	Moisture (g/100g)	pH
Village 1	84.2 (1.34)	2.38 (0.28)	1.30 (0.10)	32.3 (1.86)	12.4 (0.61)	6.22 (0.15)
Village 2	85.0 (1.47)	2.33 (0.36)	1.22 (0.17)	36.7 (2.11)	12.9 (0.60)	6.17 (0.22)
Village 3	85.2 (1.80)	2.40 (0.24)	1.15 (0.19)	40.9 (2.97)	12.7 (0.71)	6.28 (0.17)

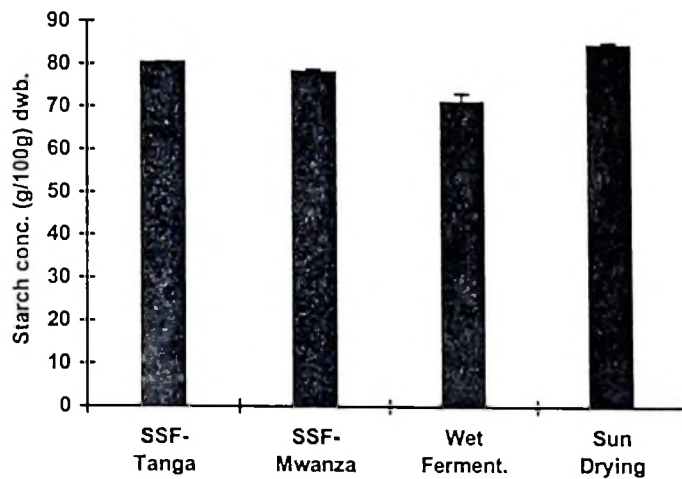
\* Values are an average of six samples. Standard deviation values are in brackets.

An average starch content of solid-state fermented cassava flour samples from Tanga and Mwanza were 80.2 g/100g dwb, and 78 g/100g dwb respectively. Comparing these values to the reported starch content of fresh cassava which ranges between 73.7-84.9 g/100g dwb (Rickard and Behn, 1987; Rickard *et al*, 1991; Asiedu, 1992) it can be concluded that solid-state fermentation had very little effect on starch content of these cassava flour samples.

Wet fermentation showed lower values of starch content, (71.5 g/100g dwb) compared to samples processed by solid-state fermentation ( $P \leq 0.05$ ).

Fermentation of cassava can cause reduction of starch content of up to 15-25% (Daubresse *et al*, 1987; Antai and Mbongo, 1994; Birk *et al*, 1996). Khetapaul and Chauhan (1990) also observed reduction of starch content in pearl millet. They said that starch degradation is caused by amylolytic action of micro-organisms in the fermenting mixture. Similar reductions of starch content of food legumes during fermentation were reported by Zamora and Fields (1979) and Odunfa (1983).

The average reducing sugars content of cassava flour samples processed by different methods are shown in Figure 4-2. Samples processed by solid-state fermentation in both Tanga and Mwanza regions showed higher reducing sugars content than that of fresh cassava reported in literature. Several workers including Gomez *et al* (1984), Asiedu *et al* (1989) and Muzondo and Zvauya (1995) reported the reducing sugars values in fresh cassava ranging between 1.9-2.3 g/100g dwb. From these results it can be seen that solid-state fermentation caused an increase of reducing sugars content in cassava samples. The increase in reducing sugars is due to hydrolysis of starch by the micro-organisms. This agrees with the results obtained by Daubresse *et al* (1987),



**Figure 4-1 Starch content of cassava flour samples processed by different methods**

Mean results from 3 villages within each region (18 samples)

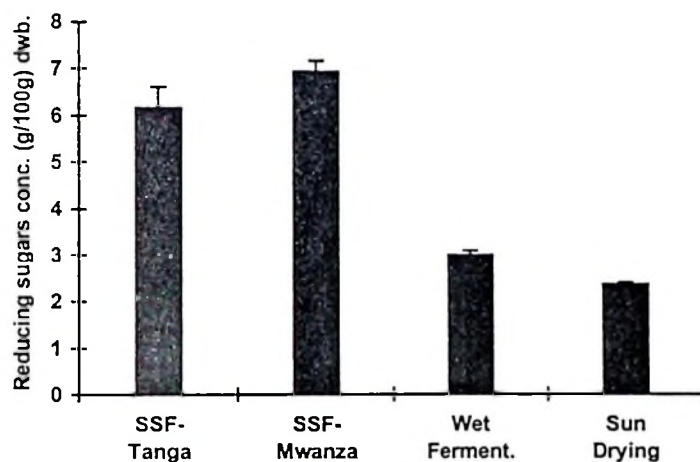
Error bars derived from S.D. values

SSF-Tanga - Solid State Fermentation in Tanga region

SSF-Mwanza - Solid State Fermentation in Mwanza region

Wet-Fermentation - Wet Fermentation in Tanga region

Sun drying - Sun drying in Kagera region



**Figure 4-2 Reducing sugars content of cassava flour samples processed by different methods.**

Mean results from 3 villages within each region (18 samples)

Error bars derived from S.D. values

SSF-Tanga - Solid State Fermentation in Tanga region

SSF-Mwanza - Solid State Fermentation in Mwanza region

Wet-Fermentation - Wet Fermentation in Tanga region

Sun drying - Sun drying in Kagera region

Zvauya and Muzondo (1993) and Muzondo and Zvauya (1995) who reported an increase of about 40%.

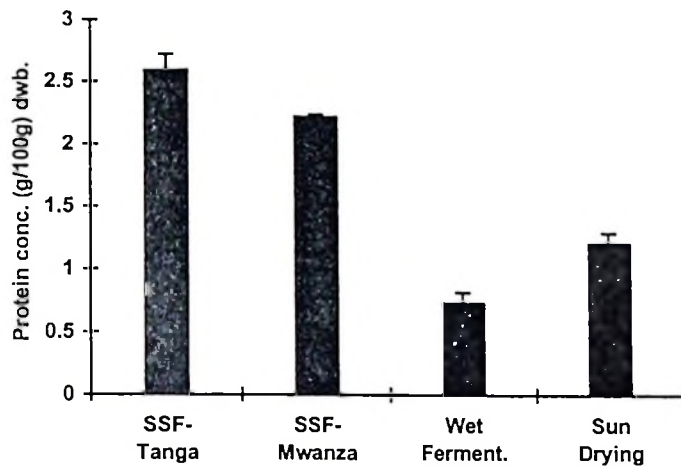
Samples processed by wet fermentation had lower reducing sugars values compared to those processed by solid-state fermentation ( $P \leq 0.05$ ) (Figure 4-2). Reduction of reducing sugars by wet fermentation was reported by Birk *et al* (1996) (28%). During fermentation micro-organisms produce polysaccharide and pectin degrading enzymes which help to lyse the cell membranes and alter their integrity. These changes are likely to leach out the soluble nutrients (i.e. reducing sugars) from cassava (Mathew George *et al*, 1991).

Low content of reducing sugars is a good attribute, since cassava flour is finally converted into 'ugali', the process which involves heating. Heat has been known to adversely affect amino acids availability through the Maillard reaction, and this is aggravated by the presence of reducing sugars (Warmbier *et al*, 1977; Adewusi and Oke, 1984; Adewusi *et al*, 1991; Knipfel *et al*, 1995).

Sun drying did not seem to have a measurable effect on reducing sugars content of cassava flour samples. This can be compared to a study conducted by Ayankumbi *et al* (1991) in which no changes in reducing sugars content of sun dried cassava flour (lafun) were observed.

Figure 4-3 shows the protein content of cassava flour samples processed by different methods.

The results show higher values of protein content in cassava flour samples processed by



**Figure 4-3 Protein content of cassava flour samples processed by different methods**

Mean results from 3 villages within each region (18 samples)

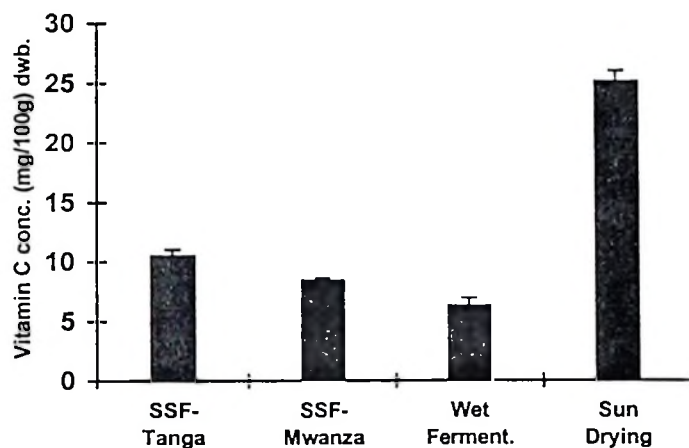
Error bars derived from S.D. values

SSF-Tanga - Solid State Fermentation in Tanga region

SSF-Mwanza - Solid State Fermentation in Mwanza region

Wet-Fermentation - Wet Fermentation in Tanga region

Sun drying - Sun drying in Kagera region



**Figure 4-4 Vitamin C content of cassava flour samples processed by different methods**

Mean results from 3 villages within each region (18 samples)

Error bars derived from S.D. values

SSF-Tanga - Solid State Fermentation in Tanga region

SSF-Mwanza - Solid State Fermentation in Mwanza region

Wet-Fermentation - Wet Fermentation in Tanga region

Sun drying - Sun drying in Kagera region

solid-state fermentation compared to those processed by other methods. The protein content of fresh cassava reported by Favier (1971), FAO (1972), Cock (1985), Ayankumbi *et al* (1991) and Muzondo and Zvauya (1995) ranges between 1.0-3.5 g/100g dwb. Solid-state fermentation causes an increase in protein content of cassava products (20%) because micro-organisms utilise starch in cassava to produce protein (Eka, 1986; Antai, 1990; Zvauya and Muzondo, 1993; Soccol *et al*, 1994; Muzondo and Zvauya, 1995). However this requires the nitrogen source.

The increase of protein content by solid-state fermentation has been reported for other food products. Joshi and Sadhi (1996) reported an increase in protein content of apple pomace during solid-state fermentation while Krishna and Chandasekaran (1995) observed an increase of protein content in cabbage processed by solid-state fermentation. Eka (1979) reported an increase in protein content of fermented sweet potatoes and rice over that of unfermented samples.

Wet fermentation seems to have caused reduction of protein content of cassava flour samples. Wet fermentation is known to cause losses of protein (Ezeala, 1984; Bokanga *et al*, 1988; Ayankumbi *et al*, 1991; Padmaja *et al*, 1994). Favier *et al* (1971) reported protein losses between 50-87% of original protein value. Padmaja *et al* (1994) said that decrease in protein content is likely to result from leaching into steeping water.

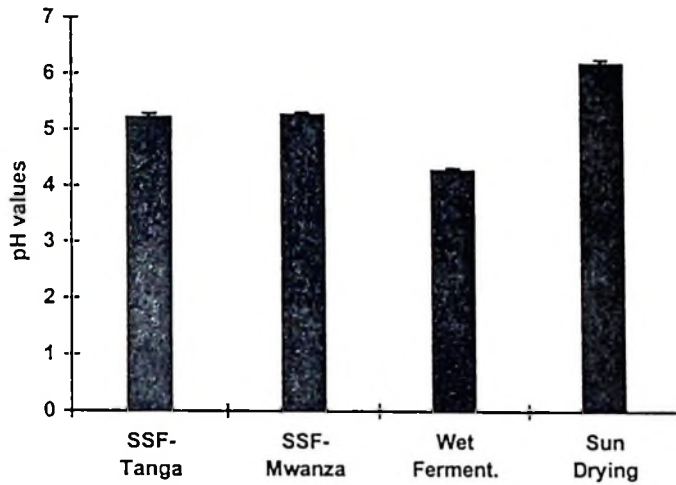
Samples processed by sun drying had protein content values lower than those processed by solid-state fermentation, but higher than those processed by wet fermentation ( $P \leq 0.001$ ) (Figure 4-3).

Cassava samples processed by both solid-state fermentation and wet fermentation show very low vitamin C values (Figure 4-4). This shows that there is a considerable loss of vitamin C during fermentation. Fresh cassava has been reported to contain vitamin C between 82-133 mg/100g dwb (FAO, 1972; Watson, 1976; Lancaster *et al*, 1982; Cock, 1985). However high values of vitamin C in cassava of up to 201 mg/100g has been reported by Ongusua and Adedeji (1979).

Processing cassava into products such as farinha, gari and fufu reduces vitamin C content by 75% or more (Pyke, 1979; Ongusua and Adedeji, 1979; Lancaster *et al*, 1982; Ezeala, 1984; Cock, 1984). Being water soluble, vitamin C can be lost through leaching into water in wet fermentation.

Figure 4-4 also shows that sun drying causes loss of vitamin C in cassava samples. Vitamin C is not very stable and usually is destroyed during processing through combined action of heat, oxygen and light (Bender, 1966; Lingel *et al*, 1994; Adom *et al*, 1997). It has also been reported that prolonged drying time increases vitamin C losses. Drying has also been shown to reduce vitamin C content of other products like, red pepper and onion flakes (Park, 1975; Powar *et al*, 1988).

Vitamin C contents of cassava flour samples processed by solid-state fermentation and wet fermentation were significantly different ( $P \leq 0.05$ ). The vitamin C content of samples processed by sun drying was also significantly different from those processed by solid-state fermentation ( $P \leq 0.01$ ) and wet fermentation ( $P \leq 0.001$ ).



**Figure 4-5 The pH of cassava flour samples processed by different methods**

Mean results from 3 villages within each region (18 samples)

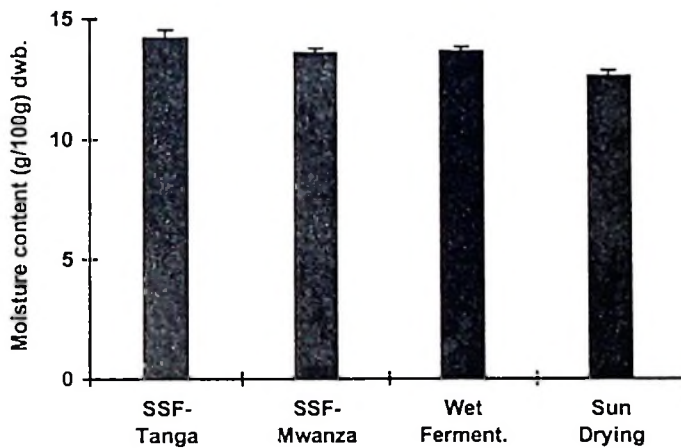
Error bars derived from S.D. values

SSF-Tanga - Solid State Fermentation in Tanga region

SSF-Mwanza - Solid State Fermentation in Mwanza region

Wet-Fermentation - Wet Fermentation in Tanga region

Sun drying - Sun drying in Kagera region



**Figure 4-6 Moisture content of cassava flour samples processed by different methods**

Mean results from 3 villages within each region (18 samples)

Error bars derived from S.D. values

SSF-Tanga - Solid State Fermentation in Tanga region

SSF-Mwanza - Solid State Fermentation in Mwanza region

Wet-Fermentation - Wet Fermentation in Tanga region

Sun drying - Sun drying in Kagera region

Figure 4-5 shows the pH values of cassava flour samples processed by different methods. Samples processed by wet fermentation had low pH values followed by those processed by solid-state fermentation, while sun dried samples had the highest pH values (Figure 4-5). All were significantly different ( $P \leq 0.05$ ). Production of organic acid (mainly lactic acid) during fermentation causes the fall in pH (Arinkele, 1967; Mathew George *et al*, 1995). Organic acids production during retting has been also reported by Ampe *et al* (1994). Fermentation imparts characteristic flavour to cassava products, which may be arising from the organic acids produced.

Fresh cassava has been reported to have pH values ranging between 6.1-6.4 (Ongusua and Adedeji, 1979; Muzanila, 1993; Giraud, 1993).

The moisture content of cassava flour samples processed by different methods is shown in Figure 4-6. The moisture content of cassava samples in all regions did not differ significantly (Figure 4-6). Even fermented samples are later sun dried to remove the water. Dried samples are stable and have a long shelf life. Fish and Trim (1993) said that inadequately dried cassava has a very limited shelf life. Gerona (1980) and Sajise and Ilag (1987) found that cassava chips dried to 11-14% moisture dwb can be stored for up to 4 months without deterioration.

In this study the moisture content of cassava flour samples ranged between 12.7-14.2% which shows that most of them were dried to the acceptable low levels although some exceeded the 14% moisture content level.

## ***Conclusion***

Variations in the composition of cassava flour within the regions were remarkably small, considering that cassava samples were from different farms, and may have encountered variation in time of planting, climate and harvesting and post harvest handling.

Cassava flour samples processed by solid-state fermentation had higher protein content and retained higher values of vitamin C compared to those processed by wet fermentation. This is beneficial to people for whom cassava is a primary or supplementary food source.

On the other hand, although wet fermented cassava samples end up with low protein and vitamin C content, referring to chapter 3, it is very effective in reducing the cyanogens content to safe levels.

Samples processed by sun drying retain higher vitamin C content, but the method is not very suitable for processing cassava varieties with high cyanogens levels.

In general, processed cassava requires adequate supplementation of protein and vitamins to meet the body requirement. The protein and vitamin content present in cassava is low, and even vitamin C which is normally found in higher amounts, it decreases during processing.

## Chapter 5

# PREPARATION AND CHEMICAL ANALYSIS OF CASSAVA FLAKES

### *Introduction*

One of the problems aggravating food shortages in developing countries is a lack of adequate, inexpensive preservation methods for local foods to ensure their supply throughout the year (Onayemi and Potter, 1974). This is especially true for cassava. Cassava is a high moisture bulky crop. It is highly perishable which causes high losses during marketing and storage.

Traditionally cassava is processed into cassava chips and granules which are later ground into cassava flour. Cassava flakes, an alternative cassava product, could be prepared by drying cassava mash on a drum drier. They are prepared in the same way as potato flakes described by several workers (Spadaro and Patton, 1961; Eskew *et al*, 1962; Deobald *et al*, 1962; Feustel *et al*, 1964; Hoover and Harmon, 1967; Spadaro *et al*, 1967; Sapers *et al*, 1974). Also some information on production of yam flakes has been published (Rodriguez-Soza and Gonzalez, 1972a,b; Ayernor, 1973; Onayemi and Potter, 1974).

Cassava flakes may have the benefits like those of yam flakes outlined by Gooding (1992), which include easy transportation, convenience food, ease of preparation as they can be reconstituted in a very short time, small shelf space and long shelf life. Cassava flakes can potentially reach a wider market, mainly because of their

convenience which could be attractive to local hotels, restaurants and Government institutions.

Drum drying, like any other heat processing, causes changes in the chemical composition of the food material, including losses of vitamins, especially where pre-cooking is involved (Onayemi and Potter, 1974; Sullivan *et al*, 1985; Wang *et al*, 1992).

The aim of this study was to prepare cassava flakes and determine the effect of processing on vitamin C, free starch, reducing sugars, protein and moisture contents, and browning (degree of non-enzymic browning).

## ***Materials and Methods***

### **Preparation and analysis of cassava flakes**

The experiment was done to determine some important parameters in producing cassava flakes (Figure 5-1). (The flakes were prepared from sweet cassava varieties).

The process involves:

Preparation of cassava, which includes peeling, washing and slicing. (1/2 x 2 x 6 cm.)-

Pre-cooking- This was done for 35 minutes at 75°C and 5 minutes at 100°C.

Cooling- Pre-cooked samples were cooled for 20 minutes.

Cooking- The cooking process took 20 minutes.

Mashing- Samples were mashe for about 5 minutes.

Drying- Drum drying took about 30 minutes .

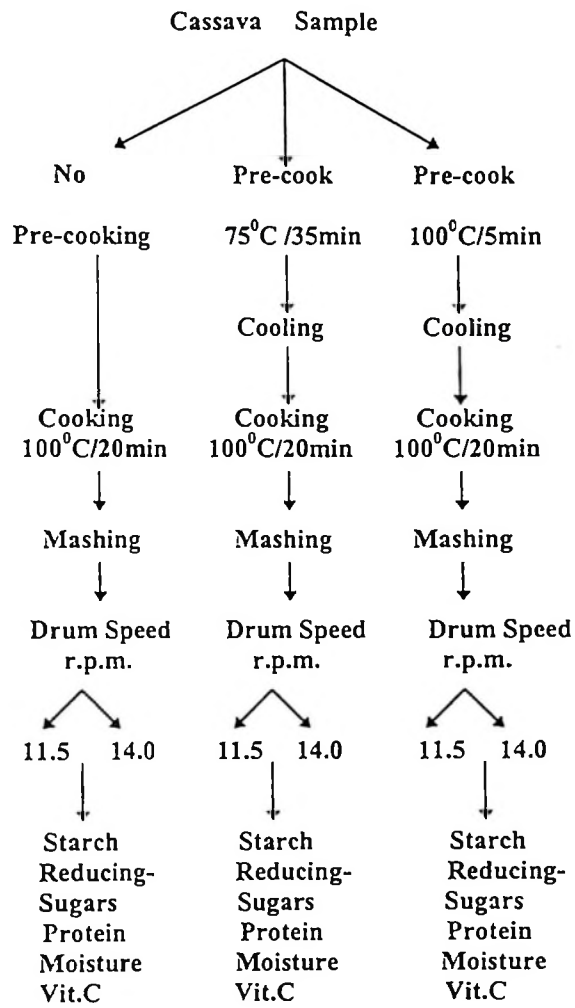


Figure 5-1 Preheating conditions and speeds of the drum drier in relation to properties of cassava flakes

The flakes were produced using a single drum drier, the effects of pre-cooking conditions and the speed of the drum drier were investigated. The flakes were analysed for their free starch, reducing sugars, protein, moisture and vitamin C content. Tests were run in triplicate, according to 3x2 factorial statistical design, with 3 replicates. The data were analysed by ANOVA. (Appendix 4).

Pre-cooking was carried out with the aim of blanching cassava roots. Minimum pre-cooking times at each temperature were determined by the peroxidase test to ensure enzyme inactivation.

### **The Peroxidase Test**

The Peroxidase test procedure was used to indicate the adequacy of blanching as described by Van Arsdel (1973) was used. In this case, the cooked cassava slices were placed on petridishes, 1 ml of 1/2% guaiacol solution was poured on the slices, then 1 ml of 0.08% hydrogen peroxide. If no colour development occurred within 3.5 minutes, the test was considered negative, indicating that the product was adequately blanched.

### **Drum drier**

The type used was a single drum drier, (Richard Simon & Sons Ltd., Basford), with 30 cm. diameter, treated by hard chrome comprising of a feed-box roller and two applicator rollers (Figure 5-2). The gap between the feed box roll and the main roll was set at 0.8 cm. The feed rollers were set with 0.025 cm. gap. The steam pressure used was 275.8 kNm<sup>-2</sup>, temperature of 140°C and drum speeds of 11.5 and 14.0 r.p.m. which correspond

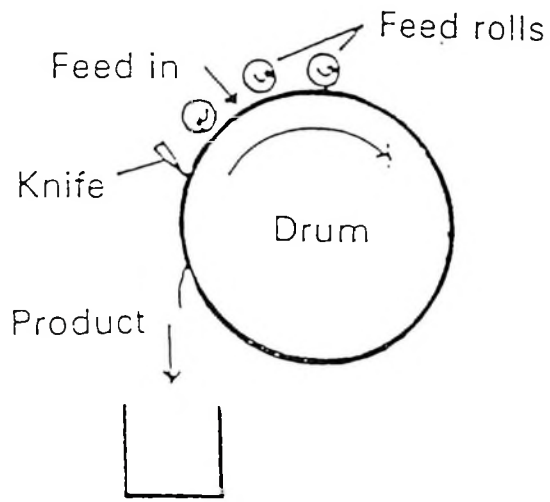


Figure 5-2 The general arrangement of the single drum drier

to residence times of 4.0 and 3.4 seconds respectively. Temperatures and drum speed used were selected following preliminary experiments. Vitamin C, reducing sugars, protein and moisture content determination was as described in Chapter 4.

### **Free starch determination**

The free starch content of cassava flakes was determined by the AOAC method (AOAC, 1984) (Appendix 2).

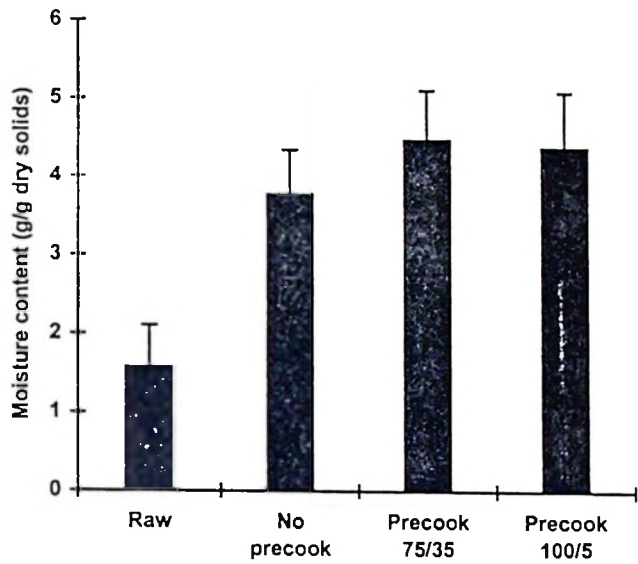
## ***Results and Discussion***

### **Cassava flakes composition**

#### ***Moisture content of cassava flakes***

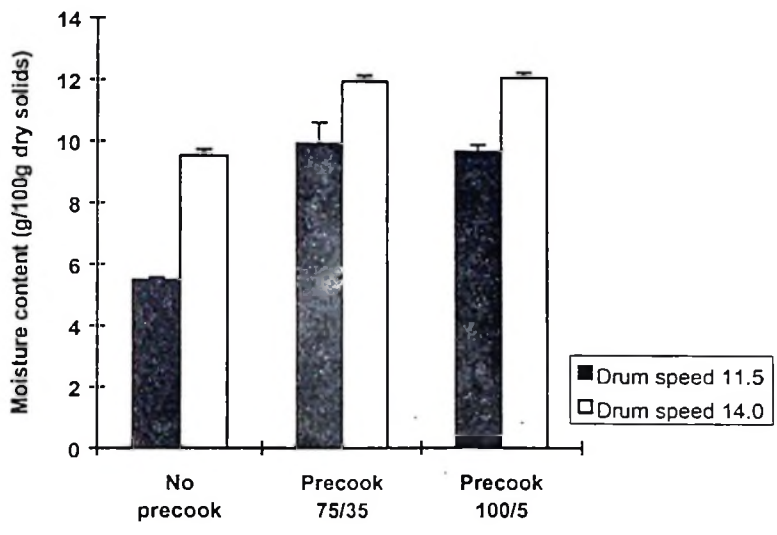
Cassava flakes produced from samples with no pre-cooking, pre-cooked at 75°C for 35 minutes and pre-cooked at 100°C for 5 minutes and dried on a drum drier at 11.5 r.p.m. (4.0 seconds) and 14 r.p.m. (3.4 seconds) were analysed for their moisture content. Also moisture contents of raw and mashed cassava samples were determined.

The cooking treatment was shown to have a significant influence on the moisture content ( $p \leq 0.05$ ) of cassava. The mash from cassava samples pre-cooked at 75°C and 100°C had the moisture content of 4.48 g/g dwb and 4.39 g/g dwb respectively. These values were higher compared to 3.78 g/g dwb for the mash from samples which were not pre-cooked ( $p \leq 0.05$ ) (Figure 5-3). This may be due to the increased hydration rate of the gelatinised starch. Pre-cooking temperature and time were not significant, as the mash pre-cooked at 75°C/35 minutes and 100°C/5 minutes did not show a significant



**Figure 5-3** Moisture content of raw and mashed cassava with and without pre-cooking

Values are means of three replicates  
 Error bars derived from S.D. values



**Figure 5-4** Effect of drum speed and pre-cooking conditions on the moisture content of cassava flakes

Values are means of three replicates  
 Error bars derived from S.D. values

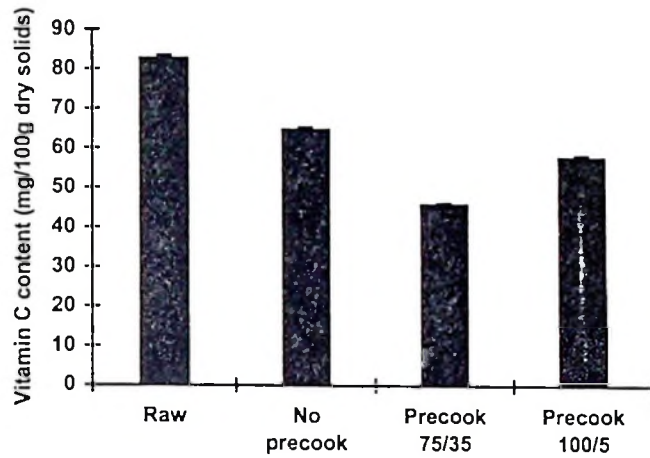
difference in their moisture content.

The moisture content of the flakes increased with increasing drum speed, and also increased with pre-cooking. Cassava flakes without pre-cooking had moisture content of 5.5 g/100g dwb at 11.5 r.p.m. and 8.3 g/100g dwb at 14.0 r.p.m. This is shown in Figure 5-4. However, flakes from cassava pre-cooked at 75°C for 35 minutes (9.93 g/100g dwb at 11.5 r.p.m.) did not show a difference in moisture content compared to those pre-cooked at 100°C for 5 minutes (9.67g/100g dwb at 11.5 r.p.m.). The drum speed and pre-cooking conditions had a significant influence on the moisture content ( $p \leq 0.01$ ).

The mash dried at higher drum speed had shorter residence time, hence retained more water. Van Arsdel and Copley (1973) reported that the diffusion of water in starch gels is very slow, especially as the moisture content is reduced. Very low drum speed is also not suitable, because it causes the sheet density of the dried mash to increase, thus, it becomes over dried on its inner surface and separates from the drum surface before diffusion of moisture from the interior of the sheet take place (Eskew, 1967; Van Arsdel and Copley, 1973).

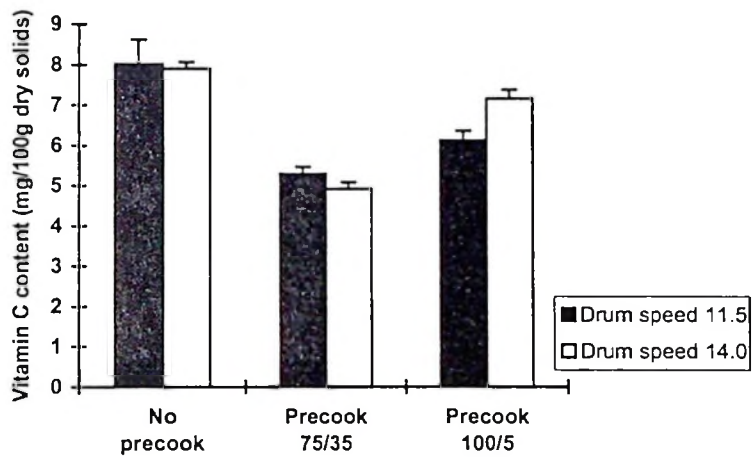
#### ***Vitamin C (Ascorbic Acid) content of cassava flakes.***

Cooking was found to cause great losses in vitamin C. Mashed cassava had lower value (64.9 mg/100g dwb) compared to raw cassava (82.7 mg/100g dwb), ( $p \leq 0.01$ ) (Figure 5-5). Jadhav *et al* (1975) reported that during mashing of potatoes, up to 57% vitamin C is lost. Pre-cooking (blanching) caused even greater losses on vitamin C. Severe blanching conditions can cause loss of vitamin C up to 50% (Benterud, 1977).



**Figure 5-5 Vitamin C content of raw and mashed cassava with and without pre-cooking**

Values are means of three replicates  
Error bars derived from S.D. values



**Figure 5-6 Effect of drum speed and pre-cooking conditions on vitamin C content of cassava flakes**

Values are means of three replicates  
Error bars derived from S.D. values

According to Olliver (1954) higher losses of vitamin C occur in blanching as the ratio of water to vegetable increases. He further said that steaming or pressure cooking causes greater losses of vitamin C, than boiling water, due to oxidation.

Cassava mash without pre-cooking lost up to 22% of vitamin C. Pre-cooking at 75°C for 35 minutes and 100°C for 5 minutes caused the loss of 44% and 30% in vitamin C content respectively. This agrees with Davidek (1990) who reported that heating at low temperature for a long time has a greater effect on vitamin C loss than heating at high temperature for a short time. Losses of vitamin C may be due to cooling of the mash before drying. Kalus and Filby (1986) observed that cooling of blanched potatoes with ambient air to 21°C led to in situ peroxide generation which enhanced the destruction of vitamin C, via radical reactions.

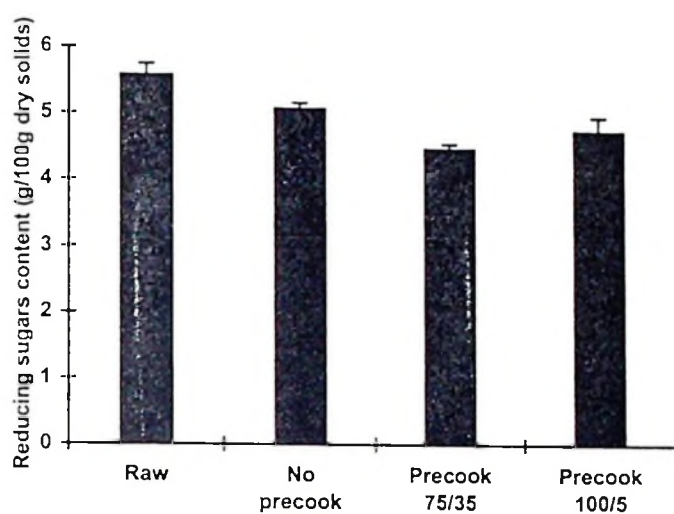
The rate of oxidative degradation of vitamin C is proportional to the concentration of dissolved oxygen in the food system. The first product of ascorbic acid oxidation, dehydroascorbic acid, is biologically active as vitamin C. However, when this decomposes hydrolytically, it forms the biologically inactive, 2,3-diketogulonic acid (Tannenbaum, 1976). Ascorbic acid can also be degraded anaerobically.

The stability of ascorbic acid varies markedly as a function of environmental conditions such as pH, concentration of trace metal ions and oxygen. It is stable in acidic medium and unstable in neutral and alkaline medium. It is also destroyed in the presence of air (oxygen), light and heat, maximum cooking losses can be up to 100% (Harris, 1971). Drum drying increased the vitamin C losses. The flakes had very low vitamin C content compared to the mash. Pre-cooking showed a significant influence on the vitamin C

content of the flakes ( $p \leq 0.01$ ). Like the trend observed in the cassava mash, flakes from the mash pre-cooked at  $75^{\circ}\text{C}$  for 35 minutes showed greater losses in Vitamin C, followed by the ones from the mash pre-cooked at  $100^{\circ}\text{C}$  for 5 minutes. Flakes from the mash without pre-cooking had higher Vitamin C retention compared to others. As shown in Figure 5-6. Jadhav *et al* (1975) said that ascorbic acid is the most difficult of the vitamins to preserve during the dehydration of foods. They reported losses of 20% in spray-dried and 30% in roller-dried milk. They suggested that addition of sulphur dioxide has a protective effect on vitamin C. Also, vitamin C can be retained by using vacuum drying. The losses during drum drying may be due to oxidative degradation of vitamin C as no antioxidants were added. Although the drum speed was shown to have a significant influence on vitamin C content ( $p \leq 0.05$ ), it did not show a definite trend because, at the same drum speed the vitamin C content increased at one pre-cooking condition and decreased at another.

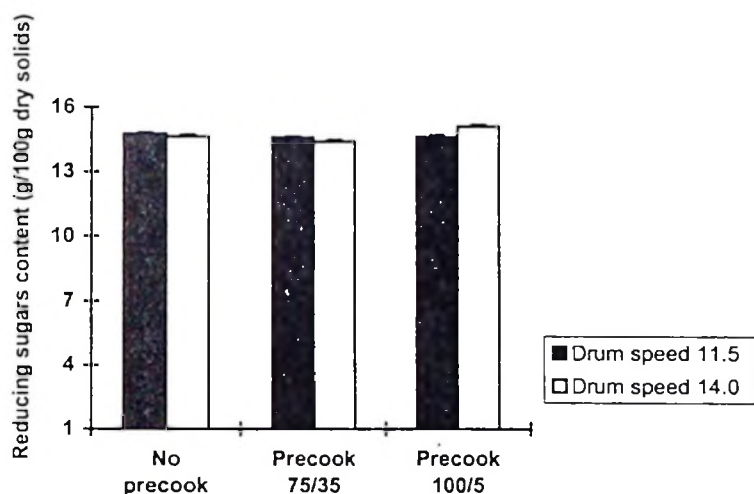
#### ***Reducing sugars content of cassava flakes.***

The reducing sugars content of raw, mashed and cassava flakes is shown in Figure 5-7 and Figure 5-8. Cassava mash showed slightly lower values of reducing sugars (5.08 g/100g dwb) compared to raw cassava (5.58 g/100g dwb). The influence of the cooking treatment here too was significant ( $p \leq 0.01$ ). This is because sugars tend to leach into cooking water. Birch (1977) explained that sugars have a strong tendency to intermolecular hydrogen bonding, which gives rise to the high solubility of crystalline sugars in water after the application of heat. In samples with no pre-cooking, the reduction of reducing sugars was small (9%) compared to pre-cooked samples (20%).



**Figure 5-7 Reducing sugars content of raw and mashed cassava with and without pre-cooking**

Values are means of three replicates  
Error bars derived from S.D. values



**Figure 5-8 Effect of drum speed and pre-cooking conditions on reducing sugars content of cassava flakes**

Values are means of three replicates  
Error bars derived from S.D. values

The means for both pre-cooking treatments were significantly different ( $p \leq 0.01$ ), with the one pre-cooked at 75°C /35 minutes losing the most of the reducing sugars.

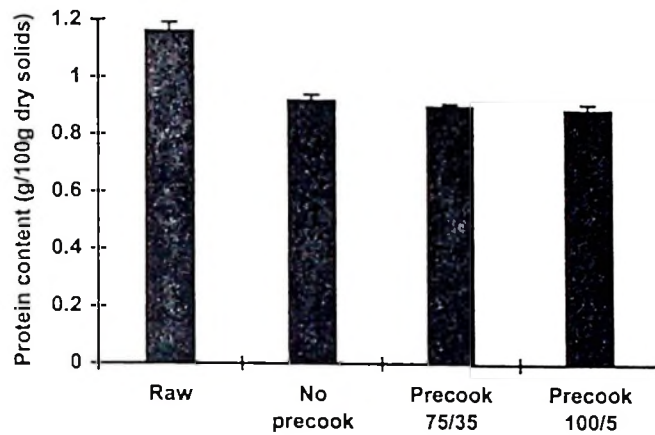
Foley and Buckley (1977) reported that during blanching of fruits and vegetables, sugar losses increase with time of blanching in water. The effect of time seemed to be more important than temperature for water blanching.

Drum drying resulted into significant gain of reducing sugars (37%). Walter and Purcell (1976) found that in potato flakes preparation, when the flakes are rapidly heated to 75°C, the natural  $\alpha$  and  $\beta$ -amylase react with the fresh gelatinised starch, decreasing the iodine blue value and producing dextrins, maltose and sugars. The influence of the drum drier speed and pre-cooking conditions did not significantly affect the reducing sugars content of cassava flakes.

#### ***Protein content of cassava flakes.***

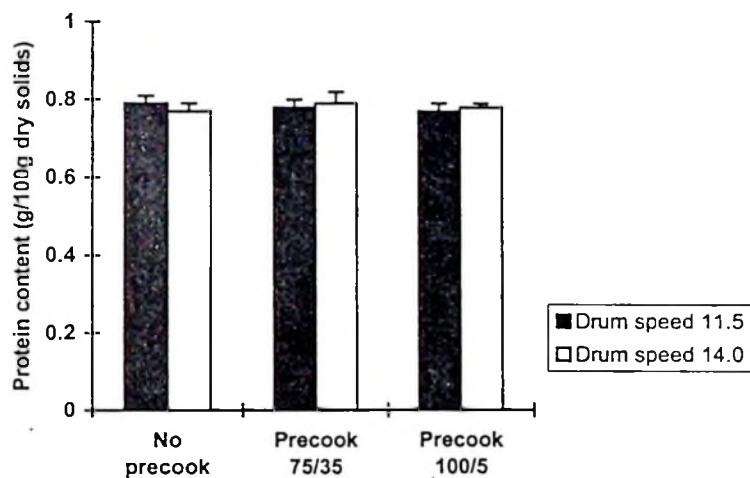
Although cooking caused losses in protein content of cassava, this was not significant. Cassava mash with no pre-cooking treatment, and the samples pre-cooked at 75°C for 35 minutes and 100°C for 5 minutes, did not show significant difference ( $p \leq 0.01$ ) in their protein content (Figure 5-9).

The drum speed (as well as pre-cooking conditions) did not significantly change the protein content of the flakes (Figure 5-10). Davidek (1990) pointed out that depending on factors like time, temperature, moisture content and reducing substances, heating may have either beneficial influence (inactivation of protein inhibitors and other



**Figure 5-9 Protein content of raw and mashed cassava with and without pre-cooking**

Values are means of three replicates  
 Error bars derived from S.D. values



**Figure 5-10 Effect of drum drier speed and pre-cooking condition on protein content of cassava flakes**

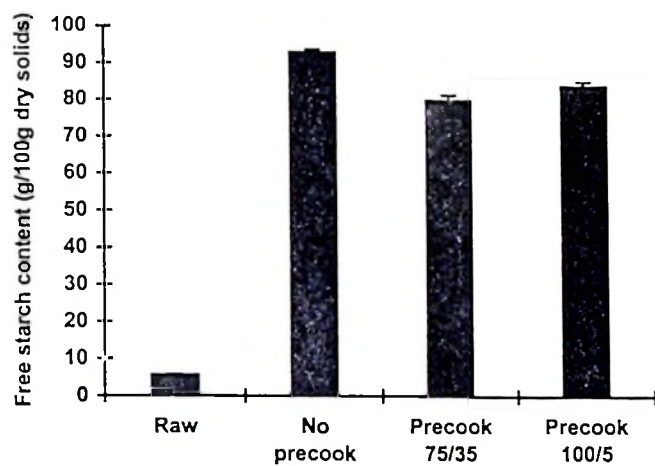
Values are means of three replicates  
 Error bars derived from S.D. values

enzymes, improved digestibility of proteins, availability of amino acids and improved flavour) or a detrimental influence (alteration of some linkages between amino acids, formation of new amino acid linkage and undesirable products). He further mentioned that heating of protein even at 100°C can cause loss of heat labile amino acids such as cysteine and lysine. Also amino acid can be lost due to Maillard reaction.

It was also mentioned that oxidation of proteins results into thioldisulphide interchange reactions, (e.g. S-S-dityrosine), and formation of degradation products, which further lowers the nutritive value of protein (Lee *et al*, 1975; Hurrell and Carpenter, 1977; Yannai, 1980; Finot, 1982; Satterlee and Chang, 1982).

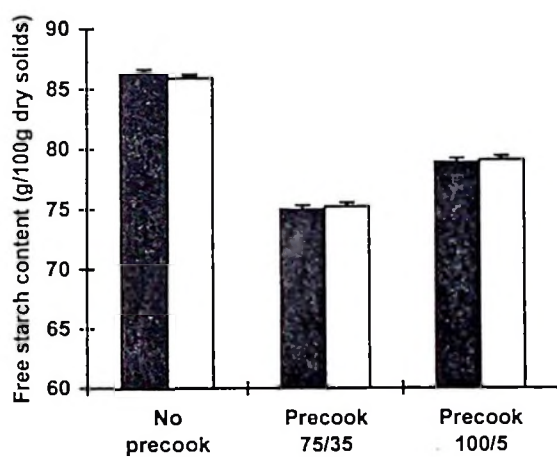
#### ***Free starch content of cassava flakes***

The influence of the cooking treatment on the free starch content of cassava mash was highly significant ( $p \leq 0.01$ ). The mash from cassava without pre-cooking step, had higher free starch content (93 g/100g dwb) compared to that from the pre-cooked samples (80 g/100g dwb) (Figure 5-11). The means were significantly different ( $p \leq 0.01$ ). Likewise, the mash from cassava pre-cooked at 75°C for 35 minutes had significantly ( $p \leq 0.05$ ) lower values of free starch content samples (80 g/100g dwb) compared to samples (84 g/100g dwb) from the samples pre-cooked at 100°C for 5 minutes. When starch is heated in water, the granules swell as they absorb water. Swelling is reversible up to the point at which the molecular structure within the granules is disrupted and birefringence is lost. Then further swelling is irreversible (at a range of temperature) and the granules undergo gelatinisation (Nielsen, 1969; Daniel and Whistler, 1985; Rickard *et al*, 1991).



**Figure 5-11 Free starch content of raw and mashed cassava with and without pre-cooking**

Values are means of three replicates  
Error bars derived from S.D. values



**Figure 5-12 Effect of drum speed and pre-cooking conditions on free starch content of cassava flakes**

Values are means of three replicates  
Error bars derived from S.D. values

When the temperature is raised above gelatinisation range, hydrogen bonds continue to be disrupted. The water molecules become attached to hydroxyl groups, starch solubility increases as well as paste consistency.

Upon cooling, starch polymers associate or retrograde, depending on the amylose concentration (Van Arsdel and Copley, 1973; Olkku and Rha, 1978). This reduces pastiness resulting from cell breakage. Potter *et al* (1959) and Reeve (1967) reported that retrogradation reduces sloughness in pre-heated potato tuber tissue. That's why the pre-cooked mash had lower free starch content because the cassava slices were cooled before the main cooking process.

The mash from cassava pre-cooked at 100°C for 5 minutes had a higher free starch content (84 g/100g dwb) than that pre-cooked at 75°C for 35 minutes (80 g/100g dwb) because, higher temperatures caused a higher degree of starch gelatinisation (Figure 5-11).

Cassava flakes had a lower free starch content (75 g/100g dwb, 75°C/35min.) compared to the mash (80 g/100g dwb, 75°C/35min.). The effect of pre-cooking showed a significant influence on the free starch content of the flakes ( $p \leq 0.01$ ). This agrees with Arthur and MacLemore (1955) who processed potato flakes using different combinations of pre-cooking and dehydrating conditions, and found that free starch decreased (8%) while reducing sugars increased (32%).

The results obtained differ from those obtained by Onayemi and Potter (1974) who dehydrated yam mash and found that free starch content of flakes was almost the same as that of the mash. Contradicting results were reported by Ayernor (1973) who found that

free starch content of yam flakes was higher than that of the mash (6%), meaning that drum drying causes an increase in free starch content. The decrease of free starch content of the flakes may be due to hydrolysis of starch to dextrans, maltose and sugars, as reported by Walter and Purcell (1976).

Cassava flakes from samples with no pre-cooking had higher free starch content compared to the ones pre-cooked at 100°C for 5 minutes ( $P \leq 0.01$ ), which also had higher free starch content compared to the ones pre-cooked at 75°C for 35 minutes ( $P \leq 0.05$ ). The drum speed did not show a significant influence on the free starch content of cassava flakes (Figure 5-12).

### ***General discussion and conclusions.***

In this study, the pre-cooking conditions had a greater effect on the nutritional composition of the flakes, than the drum speed. Pre-cooking conditions affected the free starch, moisture and vitamin C content of cassava flakes, but they did not affect reducing sugars and protein content of the flakes. On the other hand, the drum speed affected only one parameter, the moisture content. Since the heating time was found to be critical to the loss of vitamin C rather than temperature, it is suggested that pre-cooking of cassava be done at high temperature for a short time. In this case, pre-cooking at 100°C for 5 minutes is considered better than that at 75°C for 35 minutes.

Higher drum speed implies shorter residence time of the mash on the drum, so causes the end product to have higher moisture content. So in this case, the drum speed of 11.5 r.p.m. is preferred to 14.0 r.p.m. The conditions suitable from this experiment are pre-cooking at 100°C for 5 minutes and drying at 11.5 r.p.m. (4.0 seconds).

## Chapter 6

# TEXTURAL CHARACTERISTICS OF MASH RECONSTITUTED FROM CASSAVA FLAKES

### *Introduction*

Food scientists are concerned about the textural characteristics of food, mainly to meet the consumer acceptability by proper quality control of food products, also to be able to assess the advantages and disadvantages of new manufacturing processes and new foodstuffs. Both sensory and mechanical tests are useful in this subject (Bourne, 1978; Harada and Paulus, 1986; Giese, 1996; Pons and Fiszman, 1996).

Considerable attention has been paid to the textural characteristics of potato, such as stickiness of dehydrated potato and mealiness of cooked potato (Reeve, 1967; Walter and Purcell, 1976; Kabira, 1985).

The textural characteristic factors which affect reconstituted cassava flakes is stickiness. This results from release of free starch during cooking and drying. Rodriguez-Soza and Gonzalez (1972) reported that cooking time affects starch damage which is a result of starch gelatinisation. So starch contributes towards textural quality and acceptability of starchy foods (Noel *et al*, 1992).

A range of techniques may be used to monitor starch gelatinisation. These include microscopic observations of swelling or birefringence loss, amylose/iodine blue value method, differential scanning calorimetry (DSC) to determine the enthalpy change and temperature for the endothermic process of gelatinisation or by monitoring the viscosity

of an aqueous starch suspension as a function of temperature (amylograph). Normally, gelatinisation results in a large increase of volume occupied by granules, leading to a large increase in suspension viscosity (Biliaderis *et al*, 1980; Knutson *et al*, 1982; Donovan *et al*, 1983; Hosney, 1984; Lund, 1984; Leelavathi and Indrani, 1987; Noel *et al*, 1992; Erdogdu *et al*, 1995).

During the preparation of flakes, retrogradation of gelatinised starch which occurs at the cooling stage after pre-cooking of cassava, helps to reduce the stickiness of the reconstituted flakes mash. Starch retrogradation occurs when the molecules comprising gelatinised starch begin to reassociate in an ordered structure (Atwell *et al*, 1988; Ward *et al*, 1994). Although starch retrogradation is associated with staling of bread and baked goods which results in loss of consumer acceptability of the product (Kulp and Ponte, 1981), there are several examples where it has been recognised as a method for stabilising the consistency and the texture of starchy foods by encouraging development of associative bonds within the pasted starch (Ooraikul *et al*, 1974; Jankowski, 1992).

Ooraikul *et al* (1974) also reported that when a freeze-thaw technique is applied to cooked potato mash during processing of dehydrated mashed potatoes a decrease in the amount of soluble starch resulted and this led to an improvement in the consistency of the reconstituted product. Similarly, an improvement in the rheological characteristics of the dough after cooling was reported in the preparation of yam dough, (Brennan and Ayernor, 1973; Jankowski, 1992). Watanabe (1981) reported the introduction of the freeze-thaw cycle in the production of Japanese noodles (harusame) to remove their stickiness and to obtain a characteristic chewiness. In breakfast cereals, starch

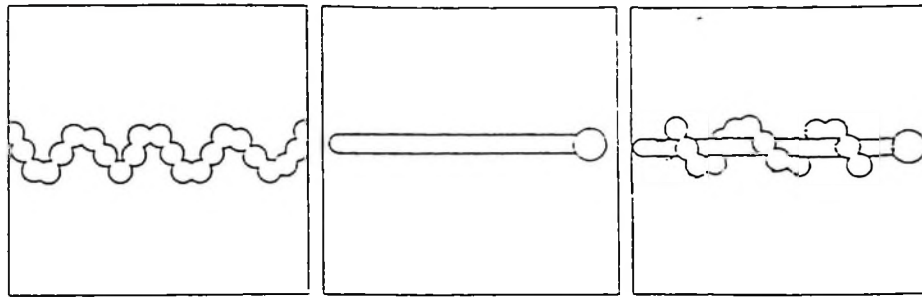
retrogradation results in an increase in the elastic modulus of the wheat grain which allows proper shredding (Jankowski and Rha, 1986).

Starch retrogradation behaviour is valuable in processes where stickiness of freshly cooked starch products prevent further mechanical processing, especially where addition of monoglycerides or surfactants which reduce the swelling and rupture of starch grains is not possible (Jankowski, 1992).

Emulsifiers have been used as amylose complexing agents in starch-based foods (Krog, 1971). According to Szczodrak and Pomeranz (1992) and Heath (1988) linear amylose has the ability to form complexes with a variety of inorganic and organic ligands, including emulsifiers. The complexes formed are heat stable and insoluble in aqueous media at pH 7. The complex forming ability of amylose with monoglycerides and related surface-active mono acyl lipids has been exploited in bread making to retard staling (Krog and Jensen, 1970). Monoglycerides have also been used in the production of dehydrated mashed potato in order to bind free amylose to control the stickiness or glueyness of the product (Krog, 1971; Hoover and Hadziyev, 1981a,b, 1982; Moorthy, 1985).

The amylose molecule is thought to wrap round the straight chain of monoglyceride (Figure 6-1). Amylose-monglyceride complexes affect other starch properties like swelling power, water binding capacity, solubility and bulk density.

In this study, the textural characteristics of reconstituted cassava flakes mash were investigated using both instrumental and sensory methods. The effect of emulsifiers on cassava starch gelatinisation characteristics, pasting properties, water absorption



*Amylose molecule*

*Straight-chain monoacylglyceride molecule*

*Amylose/monoacylglyceride complex*

**Figure 6-1 Formation of amylose-monoacylglyceride complex.**

Source: Heath (1988).

characteristics, structure of starch granules and bulk density was determined. Also the effect of cooling on retrogradation of starch was investigated.

## ***Materials and Methods***

### **Textural characteristics of reconstituted cassava flakes mash**

#### ***Instrumental Methods***

Cassava flakes were prepared with some emulsifiers to evaluate their effect on improving the textural characteristics of reconstituted cassava flakes mash.

-Pre-cooking was done at 100°C for 5 minutes.

-The emulsifiers were added during the mashing process.

-The mash was dried on a drum drier at the speed of 11.5 r.p.m. (4.0 seconds) and steam pressure 275.8 kNm<sup>-2</sup> (140°C).

Types of emulsifiers used were, stearate monoglyceride, skim milk powder and sodium caseinate. The amount added was 2% of the mash's weight except Stearate Monoglyceride which was 1.5%. Emulsifiers were chosen after preliminary trials.

The textural characteristics of the reconstituted flakes with emulsifiers and without emulsifier was determined by using Texture Profiling, (adhesiveness) and a Back extrusion method. The instrument used was the Texture Analyser. Samples were analysed in triplicate. Cassava flakes (30 g) were reconstituted with warm water (about 50-60°C). The ratio of water to flakes was 2:1. The reconstituted mash was put in small

beaker (diameter 50 mm and 65 mm height) and analysed on the Texture Analyser, using a cylindrical plunger with the diameter of 25 mm and 30 mm height. The speed of the plunger was 2.0 mm/s and distance travelled was 12 mm. The comparison of emulsifiers' effect was carried out by one way analysis of variance.

### Texture analyser

The type used in this study is TA.XT2 (Figure 6-2). The Texture Analyser is a microprocessor controlled texture analysis system. It can be coupled with PC computers and a wide range of instruments. It consists of a texture analyser test bed with probe carrier and texture analyser control console (keyboard). The Texture Analyser TA.XT2 uses a Stable Micro Systems XT.RA Dimension software which has a fully automated TPA analysis. When samples are analysed, the curves are plotted (TA.XT2. Operating manual, 1993).

Figure 6-3 illustrates a typical model profile curve obtained in texture profiling. The force-time curve obtained involves two successive 'bites' on the sample.

The height of the first peak in the cycle gives the hardness of the sample. It is expressed in chart units per unit voltage input. The ratio of the area of the second peak to the first one, gives the cohesiveness value of the sample ( $A_2/A_1$ ). Adhesiveness is measured as the area of the negative peak ( $A_3$ ). Springiness is defined as the rate at which a deformed material goes back to its undeformed condition after the deforming force is removed. It is indicated by the height that the food recovers during the time that elapses between the end of the first bite and the start of the second bite. In this work only adhesiveness was considered.

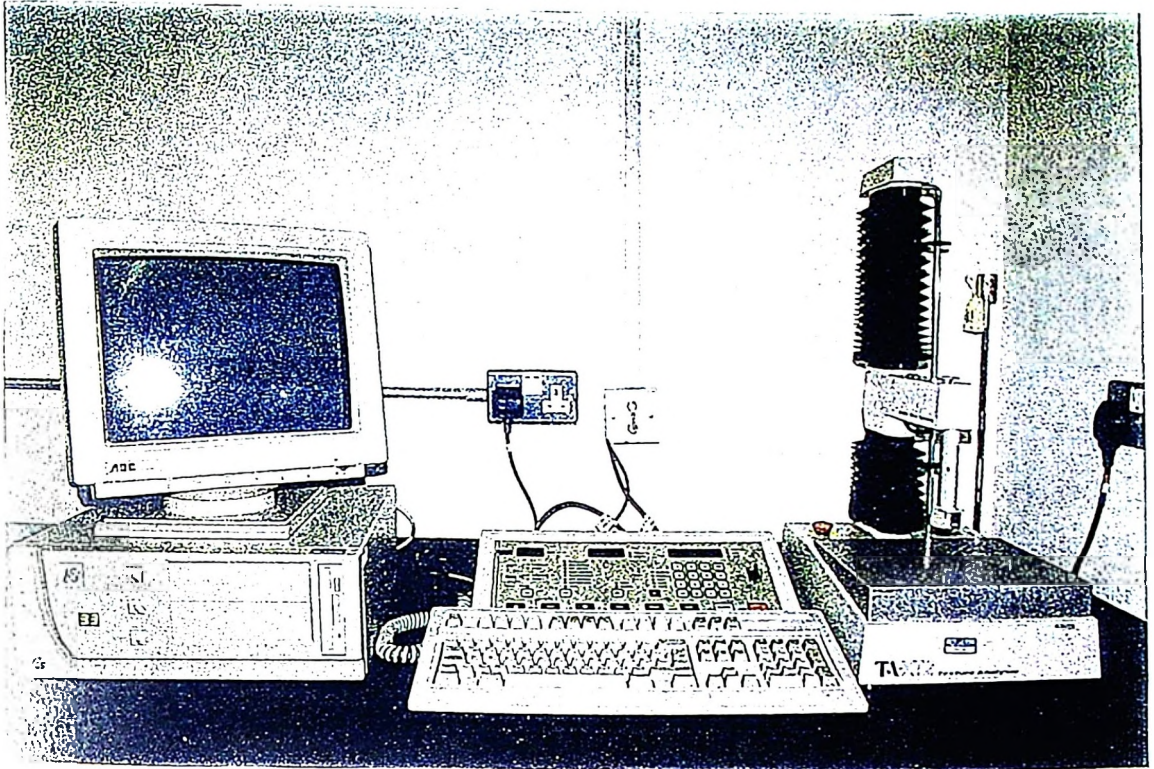


Figure 6-2 Texture Analyser. TA-XT2.

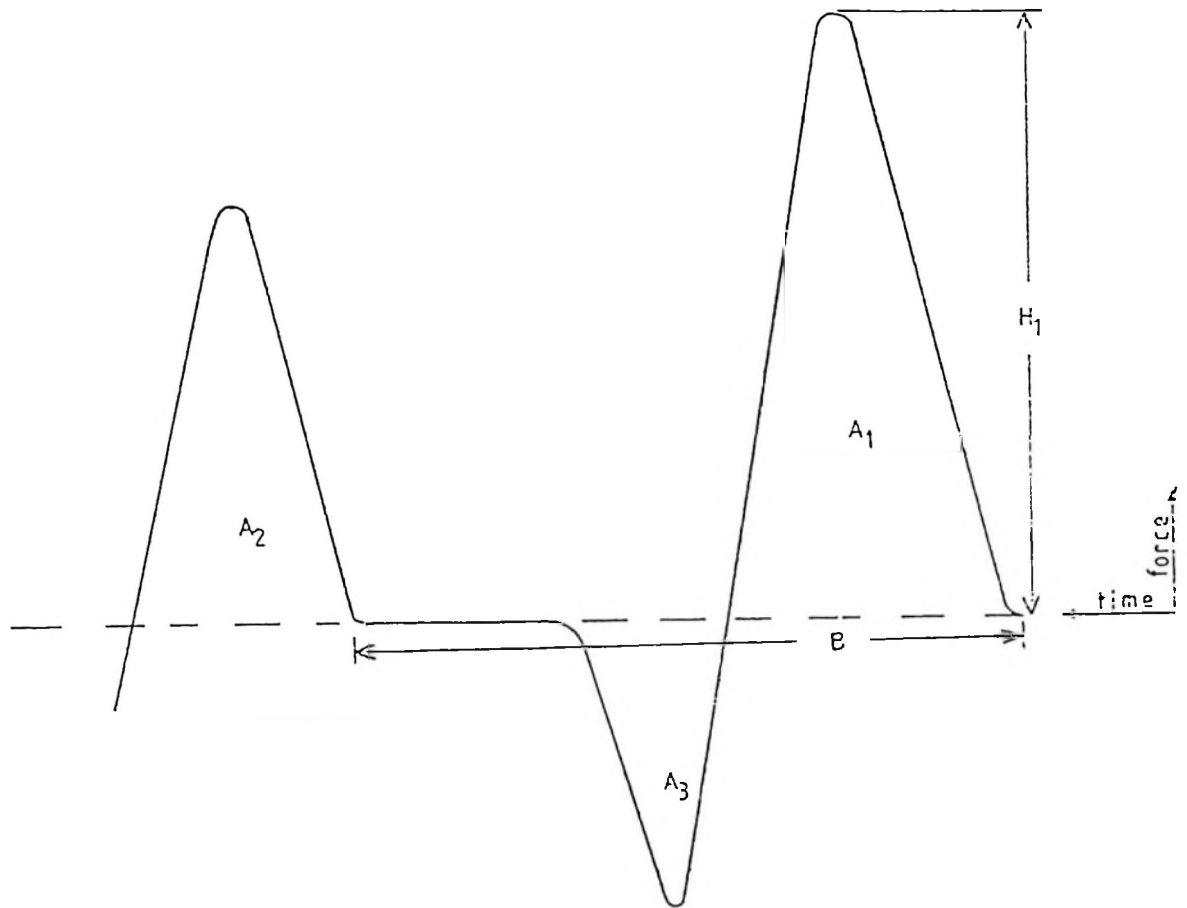


Figure 6-3 Typical Texturometer Curves

Source: Brennan *et al* (1988).

## Back Extrusion.

It was anticipated that the ease with which mash samples were extruded might be inversely related to their stickiness. Hence the Back extrusion test was performed to measure the stickiness of reconstituted cassava flakes mash. The cylindrical cell and brass plunger were used for extrusion of reconstituted cassava mash.

The cell had

-outside diameter	45 mm.
-inside diameter	30 mm.
-height (inside)	45 mm.
-Base thickness	20 mm.

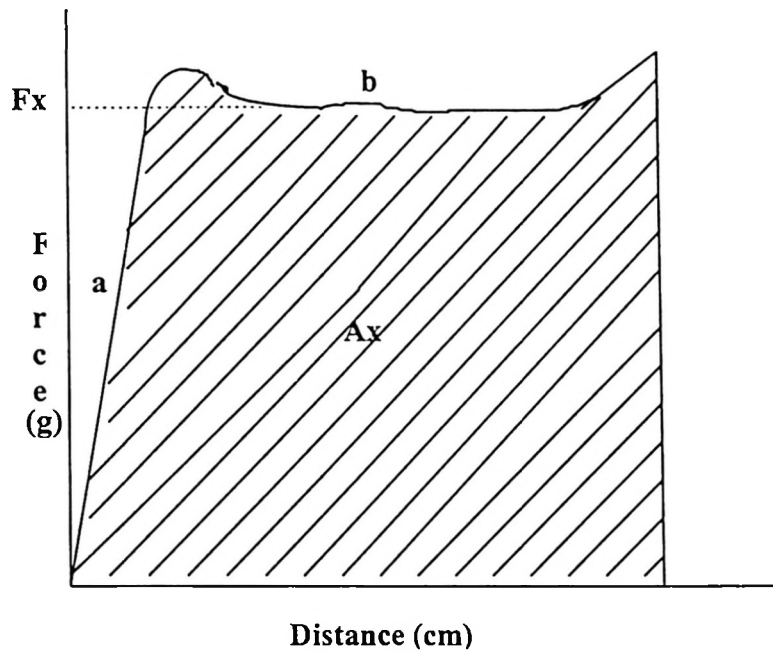
The plunger had thickness of 5 mm and 25 mm diameter. It was fixed to an attachment which allowed it to be fitted on the Texture Analyser. The speed of the plunger was 2.0 mm/s and the distance travelled was 15 mm. In this case, 50 g of cassava flakes were reconstituted in the same way as explained before.

A typical curve for force/distance for back extrusion is shown in Figure 6-4.

The area under the curve gives the total energy of extrusion.

### *Sensory analysis*

Cassava flakes samples were reconstituted with water (1:2). Cooked fresh cassava was used as the reference (standard). The samples were analysed by a sensory panel of 12 judges and the tests were repeated three times. The parameters evaluated were: stickiness, firmness in the hand and colour. Qualitative Descriptive Analysis (QDA) was used, which shows the kind or the magnitude of differences between samples. A none



**Figure 6-4 A typical curve from back extrusion test**

Source: Mohamed (1981).

a- Compression energy.

b- Equilibrium of compression force and combined shear, adhesion and extrusion forces.

Ax- Total energy of extrusion

Area below  $F_x$  - Compression forces exceed extrusion forces.

structured (100 mm) intensity line scale was used. [none (white) - extremely strong (brown)] Appendix 5.

#### **Determination of the extent of gelatinisation by soluble amylose content method.**

Evaluation of the extent of gelatinisation at different stages of flakes preparation was done by determining soluble amylose according to Wotton *et al* (1971) method (Appendix 2). Cassava flakes were prepared in the same way but varying the cooling time. (Time between pre-cooking and cooking). Some samples were cooled rapidly, others cooled for 20 min., 1 hour and 3 hours. Samples were taken at each stage, pre-cooking, cooling, cooking, mashing and drum drying and analysed for soluble amylose content.

#### **Effect of emulsifiers on starch gelatinisation by DSC method**

The effect of emulsifiers on starch gelatinisation was determined using the Differential Scanning Calorimetry (DSC) method. Cassava starch was extracted according to Moorthy *et al* (1996) method (Appendix 2).

Dry cassava starch and mash samples and suspensions were weighed into the DSC aluminium pans, on a micro balance (7-9 mg). The pans were hermetically sealed then placed in the sample chamber. An empty aluminium pan was used as the reference. Dry cassava starch and mash were heated from 20°C - 200°C at a rate of 10°C/min, while cassava starch suspensions in water were heated from 20°C - 100°C at 10°C/min.

### **Pasting characteristics of cassava flour by Brabender amylograph.**

The pasting properties of cassava flour and flakes with emulsifiers were determined using a Brabender amylograph (Brabender OHG, Duisburg, W.Germany). Cassava flour was prepared from dried cassava slices. The slices (1/2 x 2 x 6 cm) were dried in an air draft assisted oven at 50°C for 72 hours, ground into fine flour using a coffee grinder and sieved using 710µm sieve. A buffer solution was prepared from 14.8 g anhydrous disodium phosphate and 10.3 g Citric acid monohydrate made up to 1 litre with water. The amylograph was fitted with the 700 cm. cartridge, 40 g of cassava flour and flakes were weighed into a 500 ml beaker, then 46 ml of the buffer solution and 360 ml of water were added to the flour. The mixture was stirred with a plastic stirrer and the slurry poured into the amylograph bowl. The remaining content of the beaker was rinsed with a further 100 ml of water into the amylograph bowl. The amylograph was set to run with temperature change of 1.5°C/min. Samples were heated from 25°C to 95°C (AACC, 1995).

### **Water absorption characteristics of cassava flour**

The water absorption characteristics (WAC) of cassava flour samples with different emulsifiers were determined according to Onuma and Bello (1988) method (Appendix 2).

### **Bulk density determination**

The bulk density of cassava flour samples was determined according to Onuma and

Bello (1988) method. A 10 ml graduated cylinder, previously tared was gently filled with each sample. The bottom of the cylinder was gently tapped on a bench several times until there was no further diminution of the sample. The bulk density was calculated as weight of the sample per unit volume of sample (g/ml). Measurements were made in duplicate, and average results were expressed on dry weight basis.

### **Structure of cassava starch by Microscopic method**

The structure of the starch in ungelatinised and gelatinised cassava flour and cassava flakes suspensions (1%) with different emulsifiers were observed using the light microscope under phase contrast and polarised light (Olympus BH-2, Japan). The eye piece specifications were 3.3 x L.  $\approx$  x 10. The type of film used was ISO 200 Konica colour print film.

### **Colour of reconstituted cassava flakes mash with different emulsifiers measured by Hunter Lab Spectrophotometer**

The colour of flakes and reconstituted mash of cassava samples was measured using the Hunter Lab Spectrophotometer (Hunter Lab Colour Quest, Henry A. Sarasin AG, 4010 Basel). Measurements were made in the visual spectrum from 400 to 700 nm at 40 nm intervals. Data produced by the instrument were an average of four readings. Visual field used was 10°, and a D65 illuminant. Hue expressed as an angular measure (h), and chroma (C\*) were calculated as follows (CIE, 1978):  $h = \arctan (b^*/a^*)$  and  $C^* = (a^{*2} + b^{*2})^{1/2}$ . The CIELAB diagram of b\* values plotted against a\* values is represented by Figure 6-5.

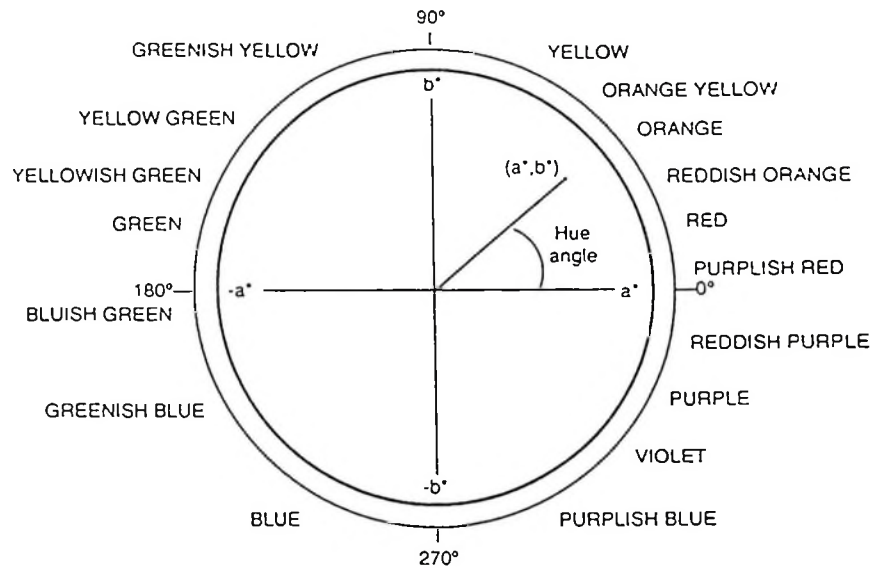


Figure 6-5 Hue sequence and hue-angle orientation on a CIELAB diagram (with ISCC-NBS color names).

Source: Voss (1992).

## ***Results and Discussion***

### **Textural characteristics of reconstituted cassava flakes mash**

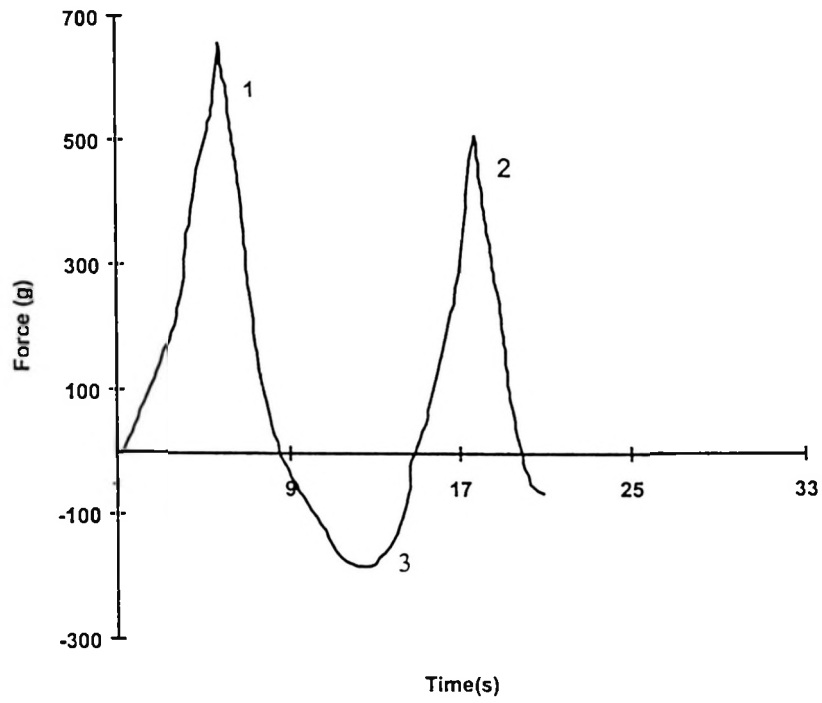
#### ***Instrumental analysis.***

##### **Texture profile analysis (TPA), (Adhesiveness)**

The typical curve from the TPA test obtained for reconstituted cassava flakes mash is shown in Figure 6-6. Reconstituted mash from cassava flakes without emulsifier, was very sticky compared to the mash from fresh cassava. This is indicated by higher adhesiveness value in Figure 6-7. The stickiness was caused by the rupture of starch granules in cassava during cooking and drum drying. Hadziyez and Steele (1979) said that when starch granules swell, the hydrated linear amylose diffuse easily through the newly formed membrane to accumulate preferentially in the external water phase.

Although the adhesiveness values of the reconstituted mash from flakes which contained sodium caseinate and skim milk powder were slightly lower compared to the one without emulsifier, (means significantly different ( $p \leq 0.1$ ), they were still sticky to the fingers.

The reconstituted mash from cassava flakes to which stearate monoglyceride was added, showed low values of adhesiveness, indicating that it was less sticky ( $p \leq 0.01$ ) compared to other samples (Figure 6-7). Van Arsdel *et al*, (1973) explained that monoglycerides are added to potato mash to improve the textural characteristics of the reconstituted mash from potato flakes. Hadziyez and Steele (1979) said that starch is complexed by monoglycerides.



**Figure 6-6 Typical curve from TPA test for reconstituted cassava flakes mash**

Area of the negative peak (no. 3) gives the measure of adhesiveness which represents stickiness.

The two emulsifiers (sodium caseinate and skim milk powder) did not show any differences in their effect on the stickiness of cassava samples. Also, they both caused the flakes to have a brownish colour. Plate 6-1 shows the colour of cassava flakes and reconstituted mash, of samples containing different emulsifiers.

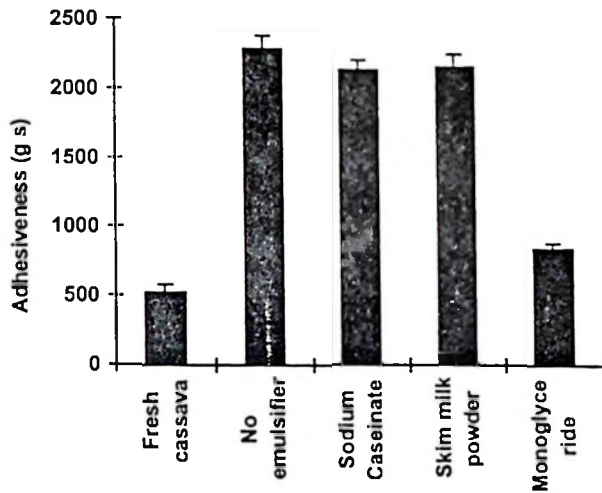
The probable cause of brown colour in the flakes with sodium caseinate and skim milk powder is non-enzymic browning, between sugars and proteins in emulsifiers. Apart from sugars in cassava, milk contains lactose. At the same time milk proteins are very rich in lysine. Finot *et al* (1981) said that when milk is heat-treated, lactose reacts with lysine to form  $\epsilon$ -deoxy-lactulosyl-lysine, which takes part in the Maillard reaction.

It was also shown by Daniel and Whistler (1981) that lipids such as triacylglycerol fats and oils, and lipid materials, such as mono- and diacylglycerol emulsifiers can form inclusion complexes with amylose. The complexes are not easily broken and resist entry of water in the granules.

The amount of stearate monoglyceride used was 1.5 g/100g of undiluted mash. Higher contents caused the mash to be somehow slippery and it did not stick to the drum drier, so the mash was not dried into flakes. Lower values produced flakes which gave a sticky mash on reconstitution.

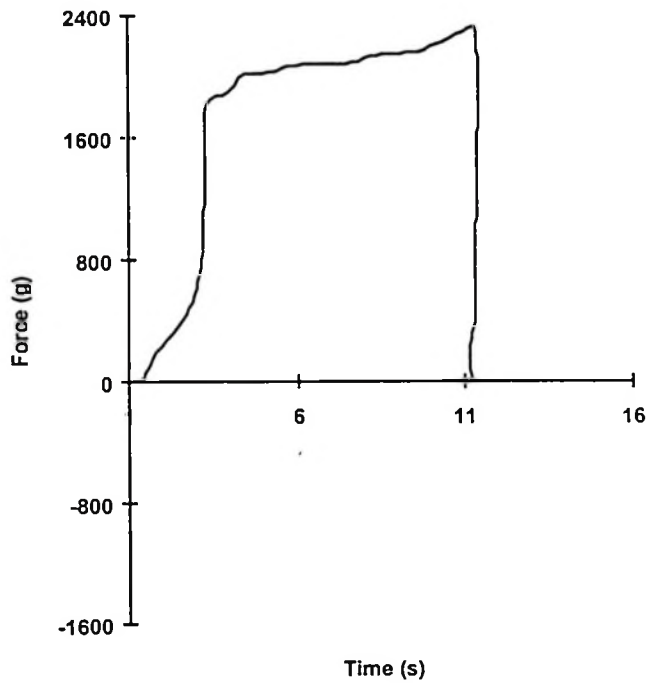
#### Back Extrusion.

The typical curve from Back extrusion test obtained for reconstituted cassava flakes mash is shown in Figure 6-8. Extrusion characteristics of the reconstituted samples were



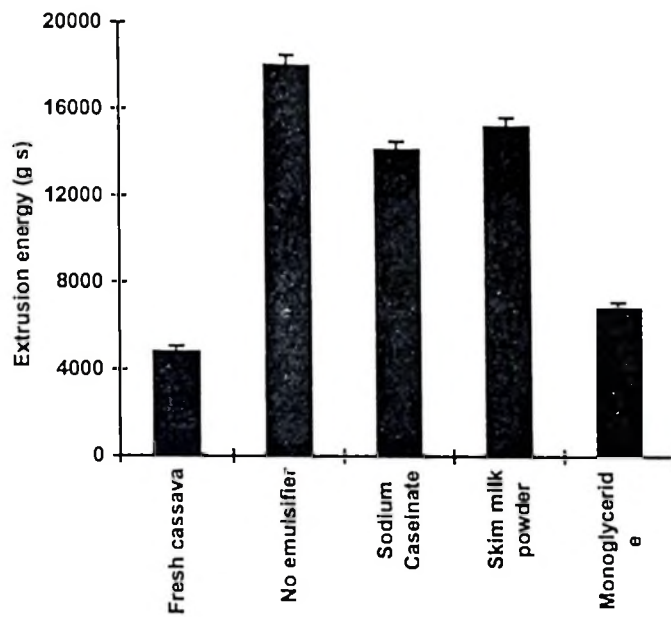
**Figure 6-7 Effect of emulsifiers on the textural characteristics of reconstituted cassava flakes mash determined by the TPA method**

Values are means of three replicates  
 Error bars derived from S.D. values



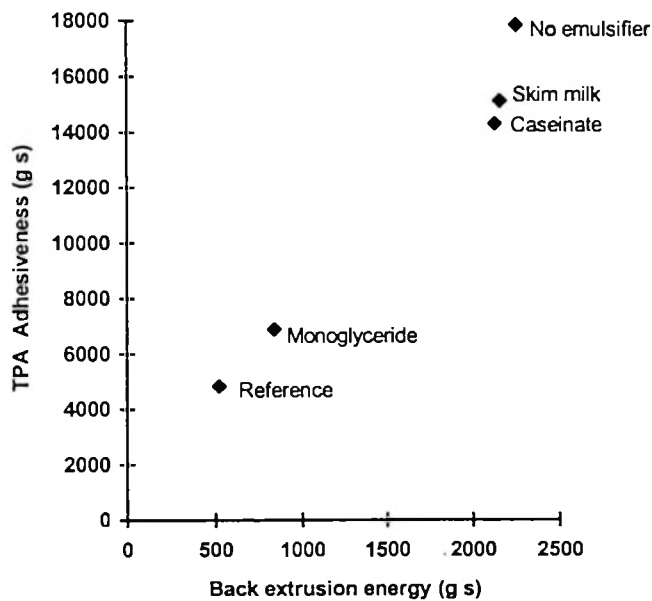
**Figure 6-8 Typical curve from Back extrusion test for reconstituted cassava flakes mash**

Area under the curve is a measure of extrusion energy which represent stickiness.

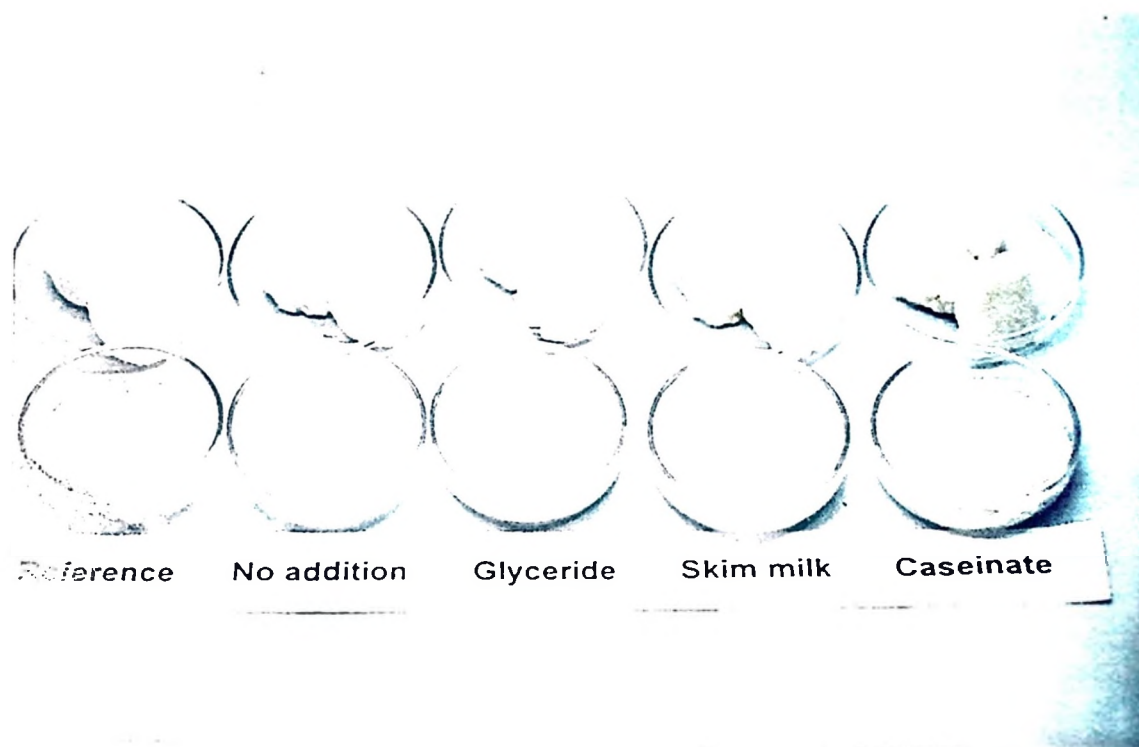


**Figure 6-9 Effect of emulsifiers on the textural characteristics of reconstituted cassava flakes mash determined by the Back extrusion method**

Values are means of three replicates  
 Error bars derived from S.D. values



**Figure 6-10 Comparison of adhesiveness from TPA and Back extrusion values**



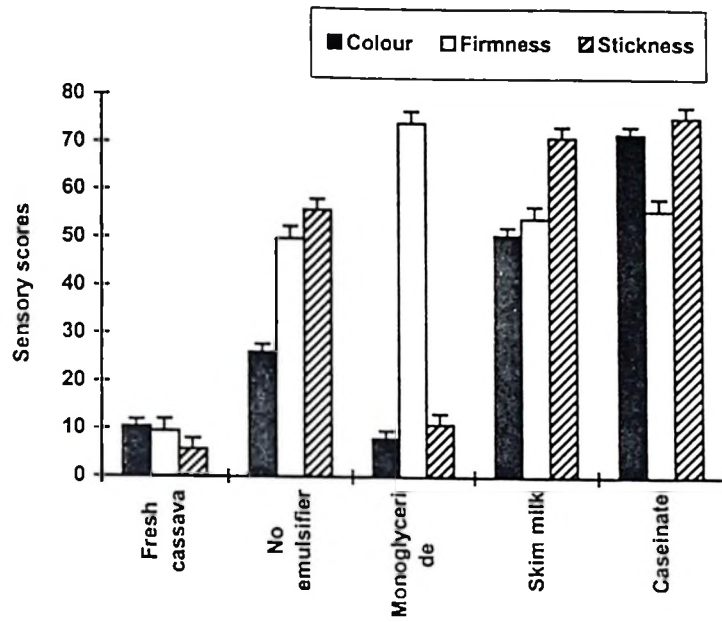
**Plate 6-1 The colour of cassava flakes and reconstituted mash samples with different emulsifiers**

related to their stickiness. High extrusion force was taken as an indicator of high degree of stickiness (large resistance to extrusion). The results showed the same trend as those obtained by the TPA test. Samples with no emulsifier added had high extrusion force values, followed by skim milk powder, sodium caseinate with stearate monoglyceride samples showing the least values (Figure 6-9). The comparison of the means showed that the difference between sodium caseinate and skim milk powder was not significant ( $P \leq 0.05$ ). Sticky samples are difficult to extrude compared to non sticky samples.

Figure 6-10 shows the scatter diagram which represents the relationship between TPA and Back extrusion tests. Fresh cassava mash and reconstituted cassava flakes mash with stearate monoglycerides had low adhesiveness values (TPA) and required low Back extrusion energy. Samples with sodium caseinate and skim milk powder, and those without emulsifiers had high adhesiveness values and also required high Back extrusion energy. This shows that both tests give similar trend ( $R^2 = 0.97$ ).

### *Sensory analysis*

Figure 6-11 shows the sensory scores of reconstituted cassava flakes mash for colour, stickiness and firmness. Scores for colour showed that samples with stearate monoglyceride were even whiter than the mash of fresh cassava ( $p \leq 0.05$ ). Samples without emulsifiers were less white compared to the mash of fresh cassava ( $p \leq 0.01$ ). Samples with skim milk powder were slightly darker, while those with sodium caseinate were darker still. The probable reason for this colour change is non-enzymic browning between sugars and amino acids in casein and skim milk during drum drying.



**Figure 6-11 Mean sensory scores of reconstituted cassava flakes mash**  
 Values are means of scores from 12 judges, each tested samples three times  
 Error bars derived from S.D. values

Firmness scores showed that, samples with stearate monoglyceride were firmer than other reconstituted samples and even firmer than fresh cassava. The mean scores for samples without emulsifiers and those with skim milk powder and sodium caseinate were not significantly different ( $p \leq 0.01$ ).

Scores for stickiness showed that the mean scores for samples with stearate monoglyceride were not significantly different from the mash from fresh cassava ( $p \leq 0.01$ ).

The results showed that samples with skim milk powder and sodium caseinate were not significantly different from each other ( $p \leq 0.01$ ) but were more sticky than the ones without emulsifier.

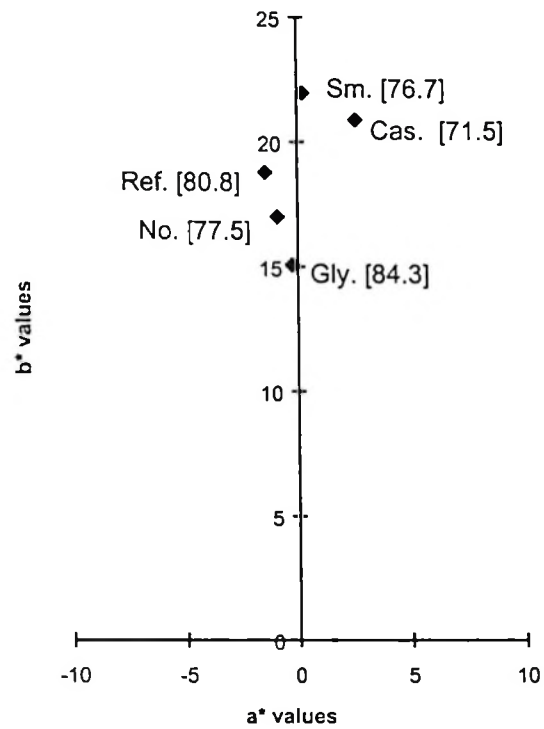
### **Colour measurement by the Hunter Lab Spectrophotometer**

Table 6-1 shows the results of colour measurement of reconstituted cassava flakes mash obtained by using the Hunter Lab spectrophotometer. This shows the  $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$  and  $h$  values when samples were measured using the Hunter Lab spectrophotometer.  $L^*$  values give a measure of lightness, the amount of incident light reflected by the sample. The greater the value, the whiter the material. Positive  $a^*$  values represent red and negative  $a^*$  values represent green, while yellow colour is represented by the positive  $b^*$  values and negative  $b^*$  values represents blue.  $C^*$  represents the chroma, which is analogous to colour saturation or intensity, and  $h$  is the hue angle (Little, 1975; McGuire, 1992; Voss, 1992).

**Table 6-1 Colour characteristics of reconstituted cassava flakes mash measured by Hunter Lab spectrophotometer.**

Colour characteristics					
Sample	L*	a*	b*	C*	h°
Reference	80.8±0.31	-1.43±0.04	18.8±0.48	18.8±0.18	94.5±0.13
Glyceride	84.3±0.38	-0.26±0.03	15.1±0.17	15.1±0.12	91.0±0.11
No emulsifier	77.5±0.50	-0.90±0.04	17.0±0.30	17.0±0.46	93.0±0.20
Skim milk	76.7±0.56	0.25±0.08	22.0±0.63	22.0±0.23	89.3±0.07
Caseinate	71.5±0.58	2.60±0.08	20.9±0.89	21.1±0.54	82.9±0.21

Values are means of four replicates



**Figure 6-12 Representation of the hue of reconstituted cassava flakes mash samples with different emulsifiers**  
 CIE-LAB a\* and b\* values are plotted on horizontal and vertical axes, respectively.

From the L\* values it can be seen that samples with stearate monoglyceride were whitest, followed by the mash from fresh cassava. Samples without emulsifiers were darker than the mash from fresh cassava. Samples with skim milk powder had low L\* values which were darker still than the mash from fresh cassava and samples with sodium caseinate which were the darkest showed the lowest L\* values. The LSD showed that means of all samples were significantly different ( $P \leq 0.05$ ).

The b\* values and a\* values were used to calculate the chroma (C\* values), and hue (h values). According to the data in Figure 6-12 and Table 6-1 the hue and chroma values show that cassava samples with sodium caseinate are dull orange yellow and those with skim milk powder are dull yellow (colour chart in Figure 6-5). Combining this with their low L\* values, shows that they underwent non-enzymic browning. The hue and chroma of all the samples with different emulsifiers were significantly different from each other ( $P \leq 0.05$ ), according to the LSD calculations.

## **Comparison of Instrumental and sensory results**

### ***Stickiness results***

The comparison of stickiness results from instrumental and sensory analysis is shown in Figure 6-13, ( $R^2 = 0.92$ ). According to the scatter diagram, both methods showed that samples with stearate monoglyceride were less sticky (closer to reference) while samples with skim milk powder and sodium caseinate showed higher degree of stickiness. It was observed that the mash from fresh cassava and samples with stearate monoglyceride, had low instrumental adhesiveness values and also scored low on the sensory scale, meaning that they were not sticky. The rest showed high instrumental adhesiveness values and scored higher on the sensory scale for stickiness.

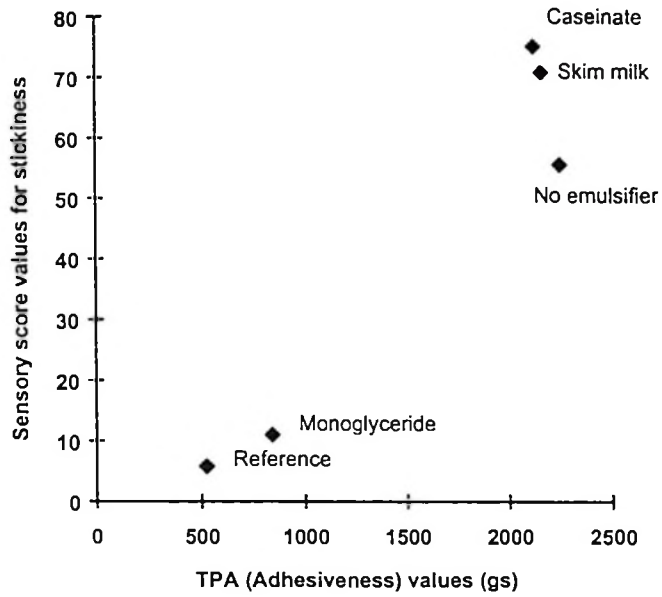


Figure 6-13 Comparison of instrumental and sensory results for stickiness (TPA)

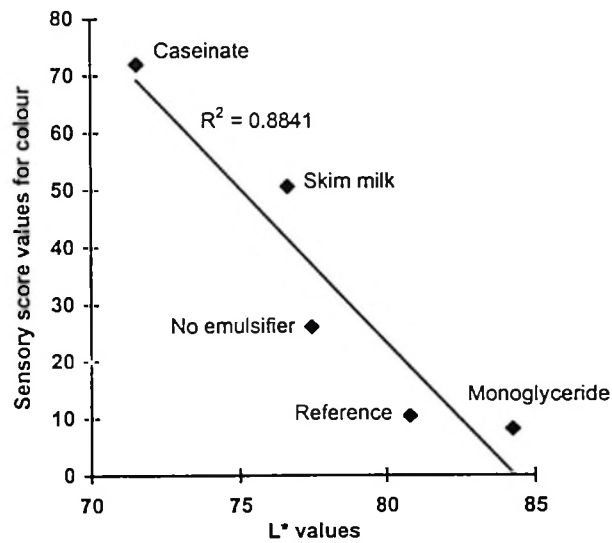


Figure 6-14 Comparison of instrumental and sensory results for colour

### ***Colour results***

The comparison of the results of sensory analysis and those of the Hunter Lab spectrophotometer are shown in Figure 6-14.

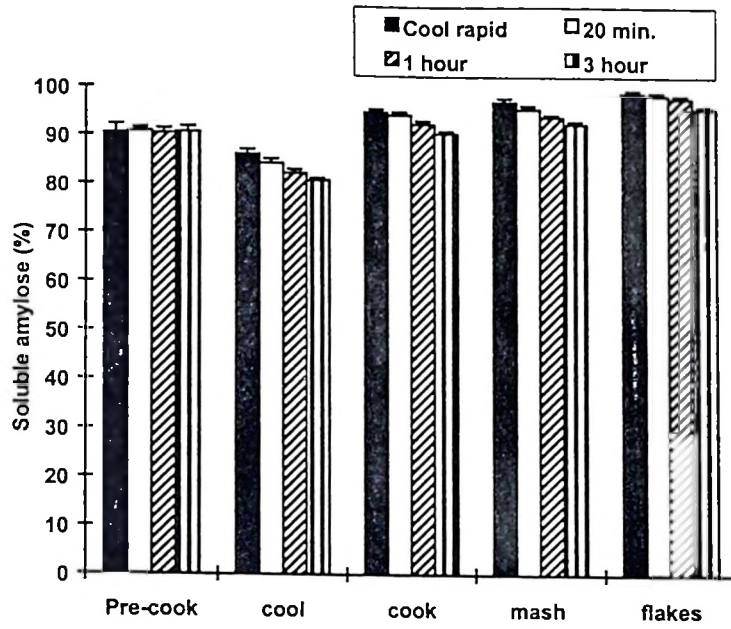
The results from instrumental ( $L^*$ ) values and sensory analysis values of colour showed negative correlation. They both indicated that samples with stearate monoglyceride were the whitest followed by the mash from fresh cassava, then samples without emulsifier. These were followed in degree of darkness by the samples with skim milk powder and samples with sodium caseinate which were the darkest.

### **Determination of the extent of starch gelatinisation using soluble amylose method**

The measure of free amylose gives an indication of the degree of gelatinisation in the sample. In this study, the soluble amylose was determined in cassava samples at different stages of flakes production. This was aimed at finding out what happens at each stage in terms of starch gelatinisation and retrogradation.

The stages involved are: pre-cooking, cooling, cooking, mashing and drum drying. Here the cooling time means the time between pre-cooking and cooking. The cooling time was varied, to establish whether cooling time has an effect on starch retrogradation. Some samples were cooled rapidly (in ice water), and others cooled at room temperature for 20 min, 1 hr and 3 hr.

Figure 6-15 shows that there was a fall in soluble amylose during the cooling stage, which rose slightly at the cooking stage, and the rest of the following stages.



**Figure 6-15 Effect of cooling conditions on soluble amylose content of cassava at different stages in cassava flakes production**

Values are means of three replicates  
 Error bars derived from S.D. values

The statistical analysis showed that, the decrease of soluble amylose on cooling the samples after pre-cooking was significant ( $p \leq 0.001$ ). Then the increase in soluble amylose content after cooking was also significant ( $p \leq 0.001$ ). Soluble amylose content increased during the mashing process ( $p \leq 0.001$ ) and again the increase in soluble amylose caused by drum drying was significant ( $p \leq 0.001$ ). However, cooling times did not have a significant effect on the soluble amylose of cassava samples ( $p \leq 0.05$ ).

The decrease in soluble amylose content during the cooling stage may be due to retrogradation of starch. Gelatinised starch tends to reassociate in an ordered structure on cooling (Atwell *et al*, 1988; Ward *et al*, 1994). Retrogradation of starch is due to reconstitution of amylose chains through hydrogen bonds (Hyun *et al*, 1988; Ferrero *et al*, 1993). Jankowski (1992) also reported a decrease of adhesiveness of cooked potato upon cooling due to association of free amylose leached from starch granules in the cooking process.

In a study on cooked potatoes conducted by Jankowski (1992), he found that the amount of soluble amylose rapidly decreased with time of storage for 8 hours and became constant after 15 hours at 4°C, while at 20°C the soluble amylose decreased for 18 hours and became constant after 24 hours.

According to Schoch and Maywald (1967) during retrogradation, starch molecules become involved in crystalline micelles which are matted together, united by molecular filaments. They further argued that although amylopectin also takes part in retrogradation, such alignment and crystallisation of their molecules is partially inhibited by their ramified structure and is limited chiefly to the outer branches.

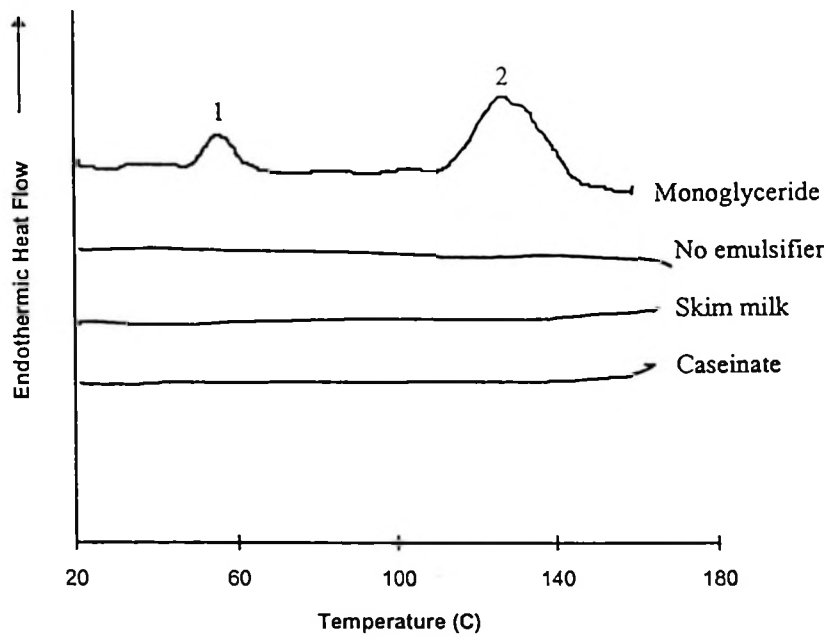
## Effect of emulsifiers on starch gelatinisation determined by differential scanning calorimetry (DSC)

When cassava starch, mash and flakes samples with different emulsifiers were analysed using the differential scanning calorimeter, samples with stearate monoglyceride thermograms showed peaks between 119°C -162°C indicating that there was starch-lipid complex formation (Figure 6-16 and Table 6-2).

Normally, the starch-lipid complex melts between 110°C-130°C (Biliaderis, 1991). In this study, the shift towards the higher temperature is because the samples were heated without water. The aluminium pans used could not withstand the water vapour pressure when heated at 200°C. In that case, emulsifiers were added to cassava mash or (starch + water) then heated in forced draft oven at 50°C to allow water to evaporate, before they were analysed by the DSC.

Wirakartakusumah (1981) heated rice starch and found that, low water-starch ratio correspond to higher gelatinisation temperature. Barichello *et al* (1991) also found that endothermic transitions of potato starch moved towards higher temperature as the volume fraction of water in starch was reduced. An increase in gelatinisation temperature as a result of low water content was also observed by Noel *et al* (1992).

Samples without emulsifier and those with skim milk powder and sodium caseinate did not show any complex formation. Heath (1988) said that stearate monoglyceride based on fully hydrogenated fats are especially suitable as starch complexing agents, due to the straight carbon chain which is easily entrapped by the helical configuration of amylose to form complexes.



**Figure 6-16 Typical DSC thermograms for cassava starch with emulsifiers heated without water at 200°C**

**Table 6-2 Temperature transitions for cassava starch, mash and flakes with emulsifiers determined by the DSC heated without water at 200°C**

Dry samples heated at 200°C	T <sub>int</sub> (°C)	T <sub>max</sub> (°C)	T <sub>end</sub> (°C)	ΔH (J/g)
<b>Starch</b>				
Starch No emulsifier	185.4±2.60	192.1±3.16	-	∞
Starch + Skim milk	181.3±1.78	185.9±1.56	-	∞
Starch + Caseinate	173.5±2.91	178.9±2.74	-	∞
<b>Starch + Glyceride</b>				
Peak 1	56.90±0.62	61.90±0.58	69.20±0.47	6.90±0.05
Peak 2	119.6±3.09	139.2±2.85	155.8±1.98	71.6±1.70
<b>Cassava mash</b>				
Mash No emulsifier	174.0±1.87	189.7±2.03	-	∞
Mash + Skim milk	165.9±1.51	169.6±1.84	-	∞
Mash + Caseinate	186.3±1.07	189.7±1.28	-	∞
<b>Mash + Glyceride</b>				
Peak 1	56.90±1.05	63.70±0.98	68.30±1.21	10.60±0.48
Peak 2	119.2±2.70	138.5±2.16	162.9±1.99	67.70±1.05
<b>Cassava Flakes</b>				
Flakes No emulsifier	173.5±1.62	176.0±2.09	187.8±2.37	125.9±1.77
Flakes + Skim milk	164.6±2.74	168.2±2.56	184.0±2.22	144.6±1.89
Flakes + Caseinate	166.4±1.75	170.2±2.67	185.2±2.01	216.3±2.42
<b>Flakes + Glyceride</b>				
Peak 1	58.50±1.01	63.10±1.23	67.60±0.91	4.240±0.31
Peak 2	119.2±2.39	136.9±2.64	157.4±2.76	67.70±0.53

Values are means of three replicates

Emulsifiers in general did not affect the gelatinisation temperature when cassava samples were heated in water at 100°C (Figure 6-17 and Table 6-3). The starch gelatinised between 61.5°C-70°C. Samples with stearate monoglyceride emulsifier had two peaks apart from the starch peak. It has been reported that amylose-monoglyceride complexes exhibit multiple melting characteristics in the DSC thermograms (Donovan and Mapes, 1980; Stute and Konieczny-Janda, 1983; Biliaderis *et al*, 1985; Biliaderis *et al*, 1986).

#### **Pasting characteristics of cassava flour with different emulsifiers determined using the Brabender amylograph**

Starch viscosity increases with gelatinisation, which is caused by swelling of starch granules. The high percentage of soluble amylose in starch may be responsible for the high viscosity values (Leelavathi *et al*, 1987; Dingat, 1984; Galvez and Resurreccion, 1993).

Figure 6-18 shows the results of the tests of the pasting characteristics of cassava flour determined using the Brabender amylograph. Samples without emulsifier and those with skim milk powder and sodium caseinate had higher viscosity ( $p \leq 0.01$ ) at 95°C compared to samples with stearate monoglyceride.

Samples with skim milk powder had higher viscosity at 95°C ( $p \leq 0.05$ ) than samples without emulsifier. Also samples with sodium caseinate showed higher viscosity ( $p \leq 0.01$ ) than the rest of the samples. This suggests that stearate monoglyceride has

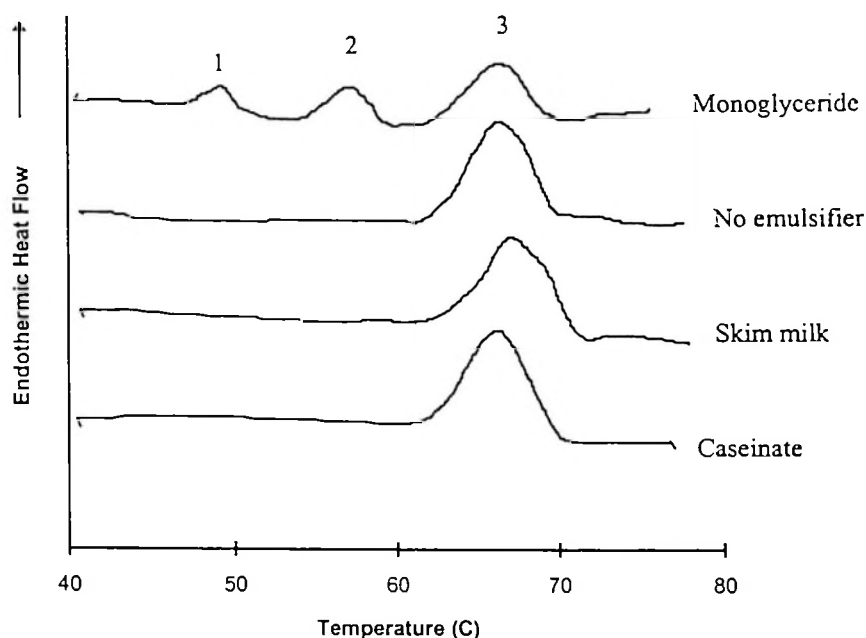
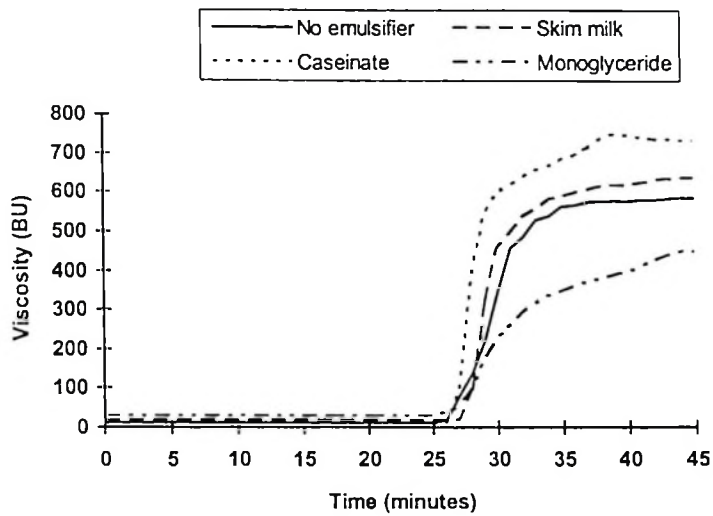


Figure 6-17 Typical DSC thermograms for cassava starch with emulsifiers heated with water (1:3) at 100°C

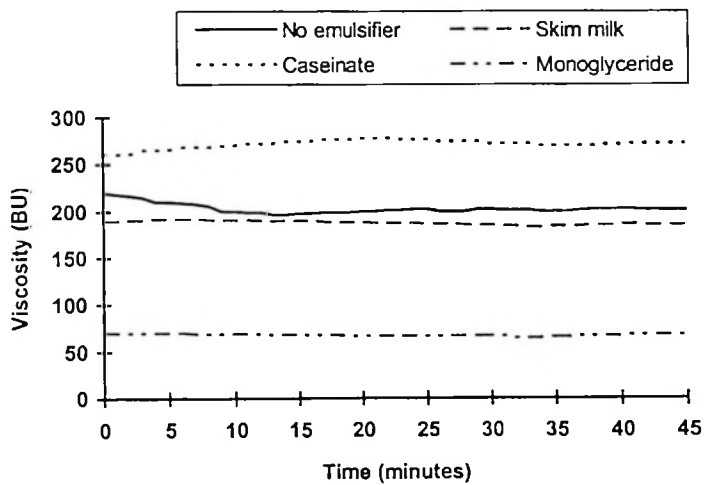
Table 6-3 Temperature transitions for cassava starch with emulsifiers by the DSC heated in water at 100°C

Samples heated in water at 100°C	$T_{int}$ (°C)	$T_{max}$ (°C)	$T_{end}$ (°C)	$\Delta H$ (J/g)
Starch + water + No emulsifier	61.5±0.91	65.2±0.54	69.7±0.48	1.5±0.15
Starch + water + Skim milk	62.5±1.12	65.8±0.86	70.1±0.94	1.3±0.09
Starch + water + Caseinate	61.9±1.25	65.0±0.88	69.4±1.32	1.1±0.11
Starch + water + Glyceride				
Peak 1	46.9±0.78	49.5±0.71	51.7±0.96	0.1±0.01
Peak 2	55.9±1.04	57.9±0.93	59.9±1.02	0.3±0.02
Peak 3	62.6±0.85	65.4±0.73	68.9±0.59	1.1±0.10

Values are means of three replicates



**Figure 6-18 Effect of emulsifiers on pasting characteristics of cassava flour by the Brabender amylograph.**



**Figure 6-19 Effect of emulsifiers on pasting characteristics of cassava flakes by the Brabender amylograph.**

reduced the soluble amylose which is responsible for high viscosity by forming starch-monoglyceride complex.

These results agree with those obtained by Hoover and Hadziyev (1981a,b, 1982) when studying the potato amylose-monoglyceride complex, and found that monoglyceride reduced swelling power and amylose solubility. Eliason (1985) said that formation of complexes prevent amylose leaching during gelatinisation, inhibit excessive swelling of starch granules when heated in water and reduce water binding capacity of starch. Jankowski (1992) and Navarro *et al* (1995) also mentioned that monoglyceride reduces swelling and rupture of starch granules.

Krog (1973) used monoglycerides to study their effect on various starches including cassava, he observed that the peak viscosity of cassava starch was reduced by the addition of monoglyceride emulsifiers. Ljubomir *et al* (1994) said that surfactant molecules can diffuse inside swollen starch granules during the pasting process and react with water soluble amylose resulting into insoluble complexes. They further said that if surfactants are introduced after the pasting process has been finished, i.e. after soluble amylose has already leached from the swollen starch granules, amylose-surfactant complexes are formed in the continuous phase.

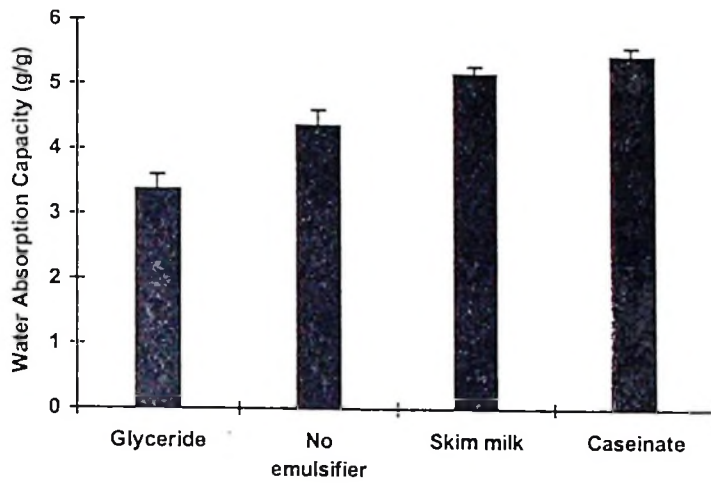
Emulsifiers did not have an effect on the pasting temperature. It ranged between 63.5°C-65°C. These results differ from those obtained by Moorthy (1995) who observed an increase in pasting temperature on addition of emulsifiers including stearate monoglyceride. Nevertheless, he observed that stearate monoglyceride reduced the viscosity of cassava starch. A change in gelatinisation temperature as a result of addition of emulsifiers was also reported by Eliasson (1986) and Buck and Walker (1988).

The Brabender amylograph also showed that cassava flakes with stearate monoglyceride had lower viscosity ( $p \leq 0.001$ ) compared to the flakes without emulsifier and those with skim milk powder and sodium caseinate (Figure 6-19). There was no peak viscosity shown because the starch in the flakes was gelatinised already. Moorthy (1985) said that addition of monoglycerides in production of mashed potato is aimed at binding free amylose to control the stickiness and glueyness of the product. So in this case also, stearate monoglyceride bound the free amylose in cassava flakes. The viscosity of cassava flakes samples with no emulsifier and those with skim milk powder was not significantly different ( $p \leq 0.05$ ). But samples with sodium caseinate had higher viscosity values ( $p \leq 0.001$ ) than the rest of the samples.

#### **Water absorption characteristics of cassava flour samples**

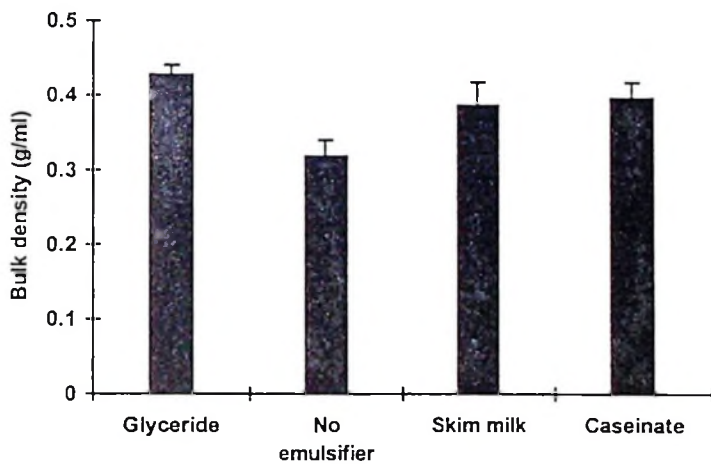
Cassava flour with stearate monoglyceride had the lowest water absorption capacity ( $p \leq 0.01$ ). Cassava flour without emulsifier and that with skim milk powder had higher water absorption capacity while flour with sodium caseinate had the highest water absorption capacity (Figure 6-20). The water absorption capacity of all cassava flour samples were significantly different from each other ( $p \leq 0.05$ ).

These results agree with those obtained by Hoover and Hadziyev (1981a, 1981b, 1982) who reported that monoglycerides suppressed the water absorption capacity of potato starch. Schoch and Maywald (1967) mentioned that amylose-complexing agents such as monoglycerides slow down hydration in pregelatinised starch, and thus improve their dispersibility. They further said that monoglycerides reduce water absorption capacity probably by binding with extracellular starch. According to Galvani *et al* (1994) and



**Figure 6-20 Effect of emulsifiers on water absorption capacity of cassava flour**

Values are means of three replicates  
 Error bars derived from S.D. values



**Figure 6-21 Effect of emulsifiers on the bulk density of cassava flour**

Values are means of three replicates  
 Error bars derived from S.D. values

Navarro *et al* (1995) lipids form complexes with amylose which form a covering on starch granules, thus reducing the capacity of starch molecules to absorb water.

These results indicate the formation of starch-lipid complex between cassava flour and stearate monoglyceride.

### **Bulk density of cassava flour samples**

Figure 6-21 shows the bulk density of cassava flour. Cassava flour with stearate monoglyceride had the highest bulk density ( $p \leq 0.01$ ) while the flour without emulsifiers had the lowest bulk density. The bulk density of samples with skim milk powder and sodium caseinate were not significantly different from each other ( $p \leq 0.05$ ) but were significantly different from samples without emulsifiers ( $p \leq 0.01$ ).

A similar trend was observed for cassava flakes too. Higher bulk density reduces paste thickness (Padmashree *et al*, 1987; Iwe and Onuh, 1992). High bulk density is of an advantage in packaging, Okezie and Bello (1988).

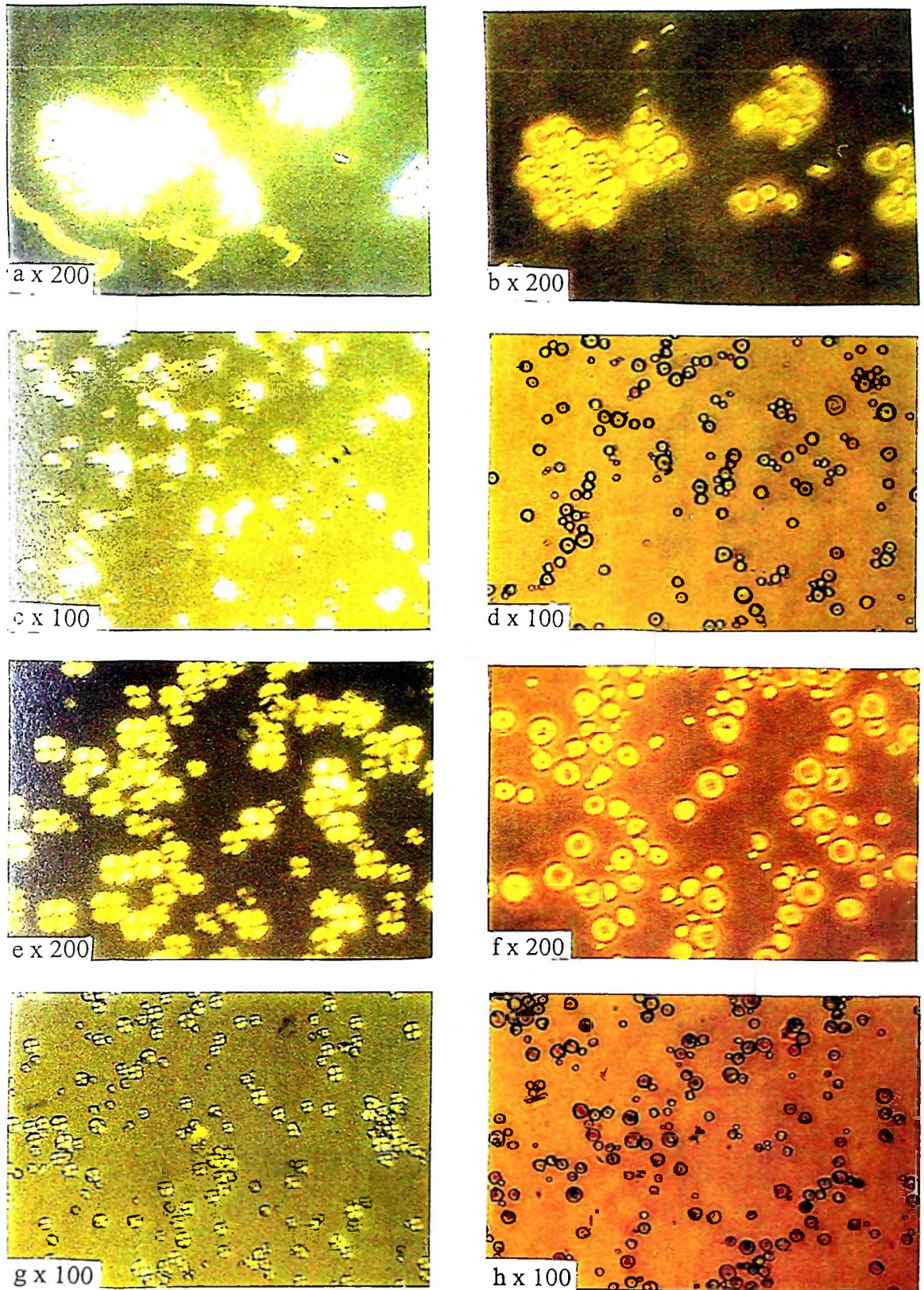
### **Structure of gelatinised and ungelatinised starch granules**

The structure of raw and gelatinised starch granules and cassava flakes with different emulsifiers were observed using a light microscope. They were observed under phase contrast and polarised light.

Figure 6-22 shows the microscopic structure of raw cassava starch with different emulsifiers. In samples without emulsifier, starch granules were found to be clumped together while in samples with emulsifiers the starch granules were dispersed in water.

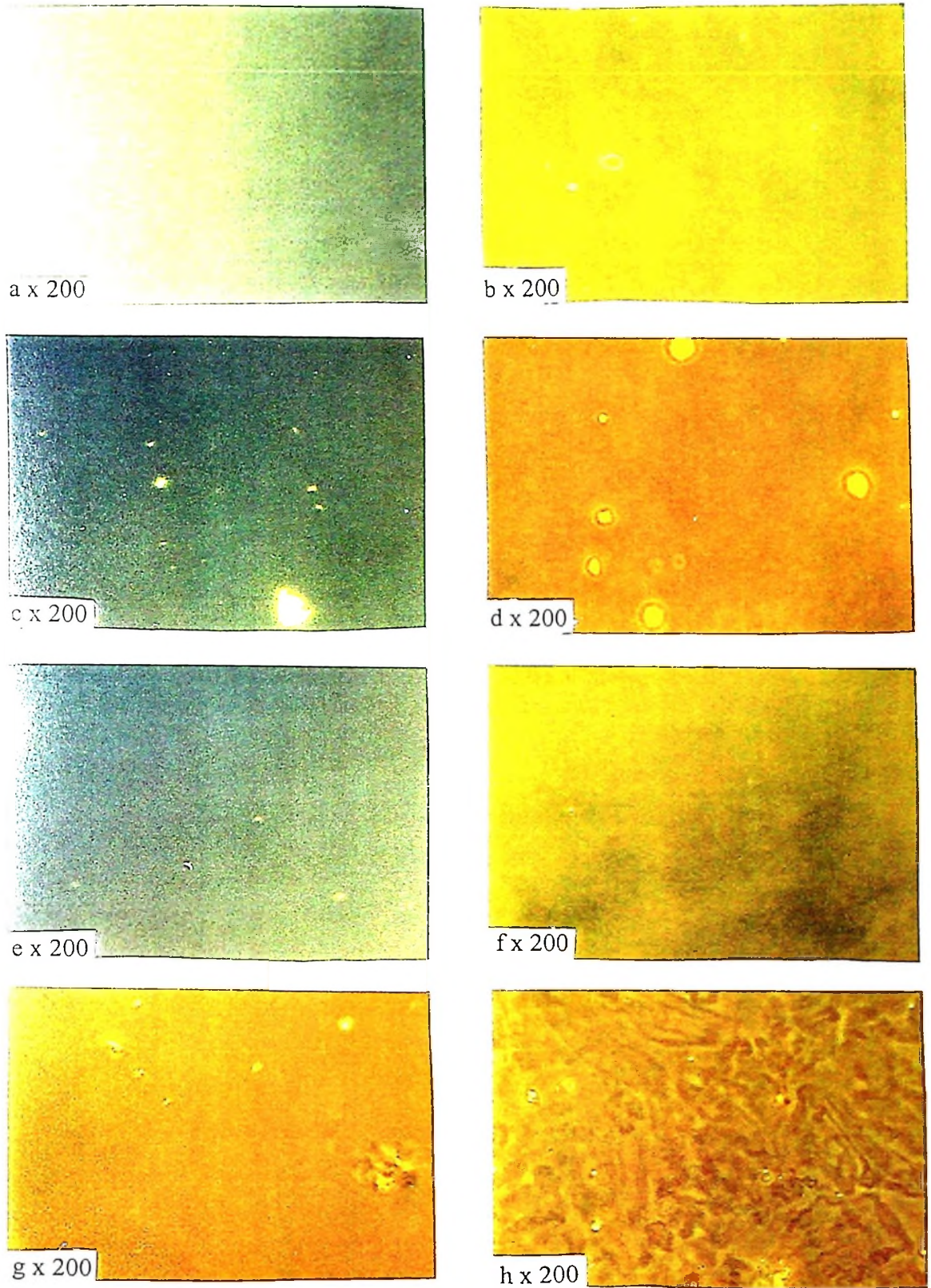
Figure 6-23 shows the structure of gelatinised starch with different emulsifiers. Raw cassava starch (10 ml, 1%) with different emulsifiers was heated (with continuous stirring) on a Bunsen burner for 1 minute, starch granules disintegrated completely and the suspension was clear (translucent). According to Figure 6-23, all starch granules in samples without emulsifier and those with skim milk powder and sodium caseinate gelatinised and disintegrated completely while samples with stearate monoglyceride although the granules burst, they did not disintegrate completely.

Figure 6-24 shows the structure of starch granules in cassava flakes prepared with different emulsifiers. This also shows that in samples without emulsifier and those with skim milk powder and sodium caseinate the starch granules burst completely. The polarised light micrographs show complete gelatinisation for samples without emulsifier and those with skim milk powder and sodium caseinate. Also, under the phase contrast they show some big particles which may be due to aggregation of gelatinised starch granules. In samples with stearate monoglyceride, starch granules did not disintegrate completely.



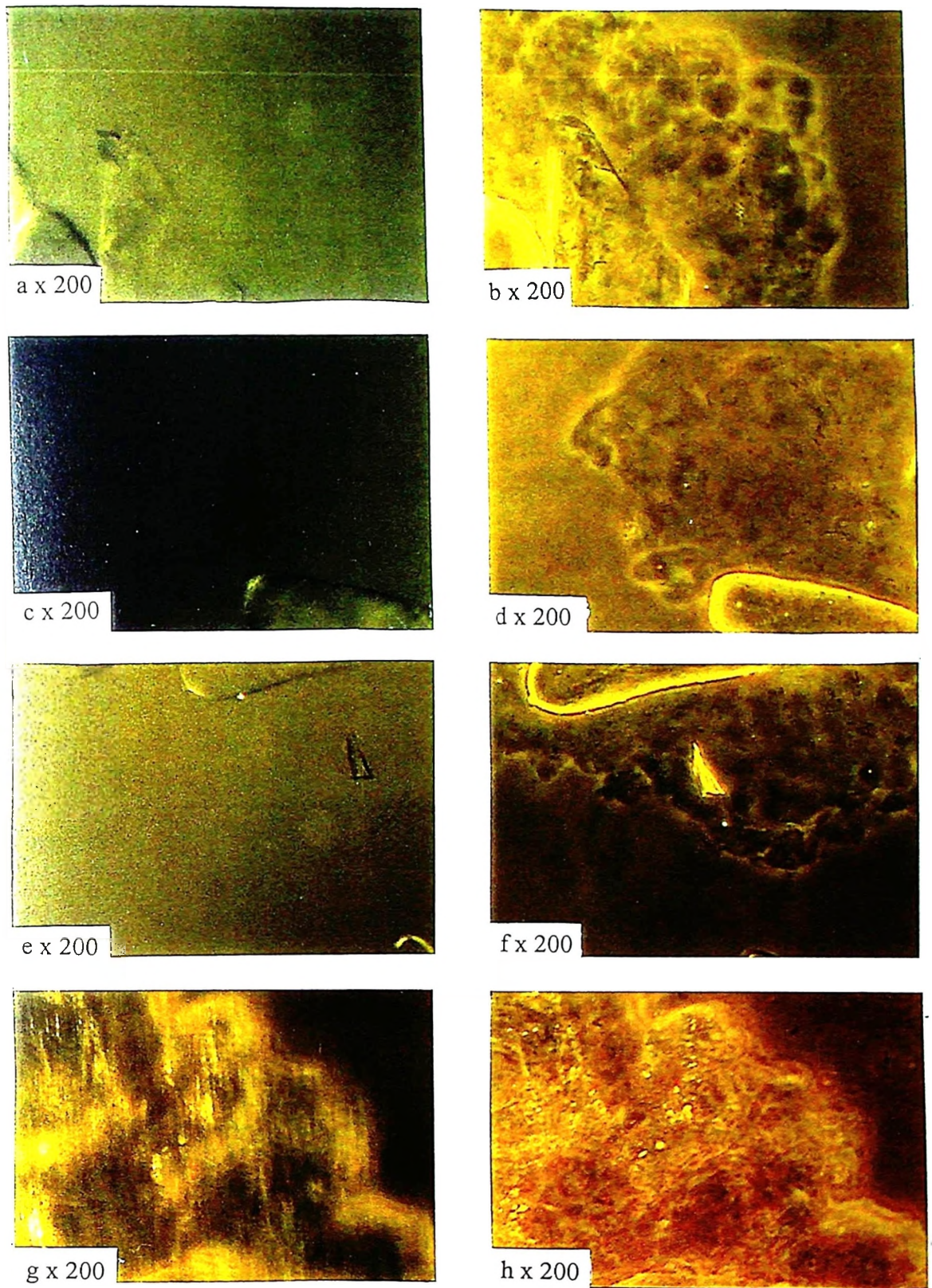
**Figure 6-22** Light micrographs of raw cassava starch granules with different emulsifiers showing the same fields under polarised light and phase contrast

**a** and **b** no emulsifier, polarised light and phase contrast. **c** and **d** skim milk powder, polarised light and phase contrast. **e** and **f** sodium caseinate, polarised light and phase contrast. **g** and **h** stearate monoglyceride, polarised light and phase contrast.



**Figure 6-23 Light micrographs of gelatinised cassava starch granules with different emulsifiers showing the same fields under polarised light and phase contrast**

a and b no emulsifier, polarised light and phase contrast. c and d skim milk powder, polarised light and phase contrast. e and f sodium caseinate, polarised light and phase contrast. g and h stearate monoglyceride, polarised light and phase contrast.



**Figure 6-24 Light micrographs of cassava flakes with different emulsifiers showing the same fields under polarised light and phase contrast**

**a and b** no emulsifier, polarised light and phase contrast. **c and d** skim milk powder, polarised light and phase contrast. **e and f** sodium caseinate, polarised light and phase contrast. **g and h** stearate monoglyceride, polarised light and phase contrast.

## ***Conclusion***

Reconstituted mash from cassava flakes becomes very sticky. Addition of stearate monoglyceride reduces stickiness. Formation of starch-amylose complex is essential to remove soluble amylose from the medium which causes stickiness. Emulsifiers which do not form complexes with amylose, i.e. sodium caseinate and skim milk powder do not improve the stickiness of reconstituted cassava flakes mash.

Mash reconstituted from cassava flakes with stearate monoglyceride had lower absorption capacity and higher bulk density compared to samples with other emulsifiers. Also the colour of the mash reconstituted from cassava flakes with stearate monoglyceride was whiter than samples with other emulsifiers.

## Chapter 7

### GENERAL DISCUSSION AND CONCLUSION

#### *Determination of Residual cyanogens and Aflatoxins in Cassava Flour*

Cyanogens limit the utilisation of cassava. Traditional methods used to process cassava have been found to be very effective in removing the cyanogens. In this study, the processes practised in some regions were effective in reducing the contents of total cyanogens to low levels. Wet fermentation gave the lowest residual cyanogen content, and average of 5.84 mg HCN.kg<sup>-1</sup> (Muheza-Tanga Region). This shows that this method is very effective in cyanogens reduction. Since cassava samples were from the bitter cassava varieties, the original cyanogens content was not less than 100 mg HCN.kg<sup>-1</sup>. A combined effect of the action of linamarase enzyme, (endogenous and exogenous from micro-organisms), and leaching of cyanogenic glucosides from cassava, reduce the cyanogens in fermented cassava. Soaking time varies from one place to another. An average of 4-6 days were reported by the farmers in the villages where samples for this study were collected.

Normally long soaking periods are employed to ensure product safety. But, it was argued that soaking for long periods of 4-5 days imparts very strong and repulsive aroma to some cassava products (Odunfa, 1985; Akingbala *et al*, 1991). However, an experiment conducted by Gidamis *et al* (1993) showed that cassava roots soaked for 3 days still retained high cyanogens levels. Soaking for a short time can only be effective in reducing the cyanogens content of cassava, if followed by pressing to remove all the water.

In general, short soaking periods lead to higher residual cyanogens, which are associated with chronic cassava toxicity and, depending to the cyanogens levels, could result into acute toxicity. In villages where cassava samples for this study were collected, farmers are aware of dangers of short soaking time through advice from the extension services.

Soaking has been reported to reduce bound cyanogens markedly. Retting which takes place in cassava root pieces, allows linamarase enzymes to act on the cyanogenic glucosides. Retting also causes softening of the roots (Lancaster, *et al*, 1982; Ayernor, 1985; Padmaja, 1995). The softening of cassava achieved during soaking also helps to reduce the labour involved in grating cassava roots.

Lactic acid bacteria associated with fermentation produce organic acid which causes the medium to have low pH. However, while low pH controls the microbiological quality of fermented cassava, it retains high cyanohydrins levels in fermented cassava, which are stable at pH 4.0 or less (O'Brien *et al*, 1992; Agbor-Egbe *et al*, 1995).

In this study, wet fermented cassava samples were found to contain low cyanogenic glucosides and high cyanohydrins levels. This suggests that most cyanogenic glucosides were hydrolysed to cyanohydrins. Samples collected were from cassava processed for home consumption which had been processed between 2-4 weeks before the samples were collected.

Harvesting is normally done in piecemeal. Farmers harvest enough for 1-3 weeks consumption. Wright *et al* (1997) mentioned the reasons for piecemeal harvesting as, labour constraints, because processing is done only by women who have to do other household work, and also lack of storage facility.

Cassava samples from Ukerewe (Mwanza region) and Tanga (Tanga district) where solid-state fermentation is practised, had relatively low cyanogens content. The main residual cyanogens were cyanogenic glucosides, while the content of cyanohydrins was very low. Although solid-state fermentation is not as effective as wet fermentation, it still reduces cyanogens to low levels. Moulds play a significant role in reduction of cyanogens. Esser *et al* (1994) reported that the moulds in solid-state fermentation of cassava include, *Rhizopus oryzae*, *R. stolonidera*, *Neusospora sitophila*, *Geotrichum candidum*.

Sun drying is said to be less effective than either wet or solid-state fermentation in cyanogens reduction. In this study the cyanogens contents of cassava from Kagera region were within the safe limits. But as mentioned earlier, these samples were from sweet cassava varieties. When bitter cassava varieties are processed by sun drying, the residual cyanogens are usually high. Piers *et al* (1974) found high residual cyanogens levels in sun dried cassava, suggesting ineffective cyanogens removal.

In the Kagera region sun drying of cassava takes about 2 days because the slices are very thin. This also reduces the labour of taking the slices out for drying every morning and taking them back into the house in the evening. In other places like Mtwara, whole cassava roots are dried without slicing. This requires a long time (7 days) to dry and normally results in lower cyanogens content compared to thin pieces. In the rainy season it can be very difficult for the whole cassava roots to dry.

During drying cyanohydrins resulting from the breakdown of cyanogenic glucosides do not accumulate. This shows that cyanohydrins are readily converted into free cyanide (HCN). Esser *et al* (1996) suggested that at higher pH (above 6.0), the presence of  $\alpha$ -

hydroxynitrile lyase and high temperature above 25°C facilitates rapid decomposition of cyanohydrins to free cyanide (HCN) which readily escapes.

In this study, the main residual cyanogens for sun dried cassava samples, were cyanogenic glucosides. The levels of cyanohydrins and free cyanide (HCN) were very low. Many workers have found the levels of residual cyanogens in sun dried cassava roots to exceed by far the recommended limit of 10 mg HCN.kg<sup>-1</sup>.

From these results it can be seen that cassava products processed by wet fermentation in the villages are safe for human consumption. If suitable processing techniques are used, the cyanogens content of cassava of the bitter varieties can be reduced to very low levels. Therefore, it appears that the cyanogenic glucosides in cassava may not limit its utilisation for food and feed. Circumstances which normally lead to cassava poisoning are famine and extreme poverty where people can be forced to eat poorly processed cassava.

No aflatoxins were found in any of cassava samples collected from different villages. mould growth does not always lead to aflatoxins production. Esser and Nout (1989) when they studied fermented cassava in Mozambique and did not detect aflatoxins in moulded cassava. Also Munimbazi and Bullerman (1996) in a study conducted in Burundi, found no aflatoxins despite the cassava being contaminated with *Aspergillus flavus*, *A. parasiticus* and *Penicillium citrium*, *P. corylophilum* and *P. chrysogenum*.

## ***Chemical Composition of Cassava Flour Samples from Tanzanian Villages Processed by Different Methods***

Processing of any kind can affect the nutritional quality of foods. In this study, the nutritional parameters analysed in cassava flour samples were starch, reducing sugars, protein, vitamin C and moisture content. The pH of the samples was also measured. The original nutritional values of cassava samples were not known but the comparison was made to the ranges reported in literature.

Hydrolysis of starch leads to the production of reducing sugars which causes its levels to rise in fermented products. In this study, cassava samples produced by solid-state fermentation had a higher content of reducing sugars than those processed by wet fermentation. These findings agree with those obtained by Daubresse *et al* (1987), Zvauya and Muzondo (1993) and Muzondo and Zvauya (1995). Wet fermentation resulted in lower reducing sugars levels, and as argued by Mathew George (1991), leaching out of reducing sugars might be one of the reasons for the low levels. Sun dried samples showed low reducing sugars contents. Ayankumbi *et al* (1991) observed that sun drying caused no change in the reducing sugars content.

The protein content of wet fermented and sun dried cassava samples was low in samples collected in Tanzanian villages. This showed the same trend as the results obtained by Padmaja *et al* (1994). They argued that protein tends to leach into steeping water during wet fermentation as a result of micro-organisms breaking down the cell membrane. Sun drying caused small changes in protein content of cassava. A slight decrease of protein in sun dried cassava was also reported by Maini *et al* (1991).

In this study, the vitamin C reported is a total measure of both ascorbic and dehydroascorbic acid. In all samples (fermented and sun dried) the retained vitamin C content was adequate to meet at least a reasonable proportion of vitamin C requirement of the body.

The moisture content of all the samples was almost the same ranging between 12-14.2% dwb. Samples with less than 14% moisture (dwb) would be stable during storage. Low moisture content gives stability to stored cassava products. Mould damage can occur when the product is stored before proper drying (Wright, 1997). When cassava roots are harvested and dried during the dry season, they dry very quickly and effectively. The samples in this study were processed in the dry season.

In general, solid-state fermentation has been found to improve the nutritional value of cassava by increasing its protein content. Sun drying resulted in high vitamin C retention. However the problem is that, these two methods may cause cassava to retain higher residual cyanogens than wet fermentation. Wet fermentation, although it causes a decrease in nutritional value of cassava, has the advantage of reducing cyanogens to low levels.

### ***Preparation and Chemical Analysis of Cassava flakes***

Instant dehydrated mashed cassava is a convenient food which may attract consumers. Drum drying which involves heating, affects the nutritional composition of cassava flakes. In this study the effect of pre-cooking and the speed of the drum drier on vitamin C, free starch, reducing sugars, protein and moisture content were investigated.

Cooking of cassava caused losses in Vitamin C. The pre-cooking process caused even higher losses. Samples cooked for a longer time, 75°C for 35 minutes had higher losses than those pre-cooked at 100°C for 5 minutes. Onayemi and Potter (1974) reported substantial losses in vitamin C in cooked yam for flakes preparation. They observed further vitamin C reductions during mashing and drying. In order to preserve vitamin C in flakes, shorter pre-cooking time is a better option.

Cooking lowered the reducing sugars content of cassava. Again the long pre-cooked cassava resulted in the lowest reducing sugars values. Leaching of nutrients may be the main reason for this reduction. The longer the food remains in water, the higher the nutrient loss. Drum drying resulted in an increase in reducing sugars content of cassava flakes. The gain was the same in all samples, regardless of the pre-cooking time and temperature. The drum speed, which reflects the residence time of the mash on the drum drier, did not affect reducing sugars and vitamin C content of cassava flakes.

The protein content of cassava was not affected by the cooking process. But a decrease in protein content was observed in cassava flakes. Neither pre-cooking conditions nor drum speed seemed to affect the protein content of cassava flakes. Protein loss during drum drying is more serious considering that cassava has a low protein content in the first place.

During processing of starchy foods, the free starch is what influences the properties of the food. Cooking of cassava increased significantly the free starch content due to gelatinisation. In this case, pre-cooked samples had lower free starch content than samples which were not pre-cooked. The cooling process which follows pre-cooking is responsible for a reduction in free starch content of pre-cooked cassava. High free starch

content is not desirable because it causes pastiness in the reconstituted cassava flakes (Onayemi and Potter, 1974; Rikard *et al*, 1991). Drying reduces free starch content. Pre-cooked samples had even lower free starch content than samples without the pre-cooking treatment. But in this case, unlike the other situations where pre-cooking at 100°C was better for retaining high vitamin C and protein content, high temperature caused higher gelatinisation rate, so caused high free starch content which is not desirable. The drum speed had no effect on free starch content.

Pre-cooking caused an increase in moisture content of cassava. Pre-cooked samples had even higher values. This is because, gelatinised starch had a higher water holding capacity (Lund and Lorenz, 1990; Prakash and Ramanan, 1995). Pre-cooking times and temperatures did not show any difference in their influence on the moisture content of cooked cassava.

Pre-cooked cassava produced flakes with higher moisture content than samples without pre-cooking treatment, but pre-cooking temperatures and times did not show any difference in their influence on moisture content of the flakes. Drum speed had an effect on the moisture content of cassava flakes. Shorter residence time of 3.4 seconds, (higher drum speed 14.0 r.p.m) caused flakes to have higher moisture content than the ones produced with longer residence time of 4 seconds (11.5 r.p.m.).

From these results, it can be concluded that pre-cooking should be carried out at higher temperature for a short time rather than at low temperature for a longer time. Also a residence time of 4.0 seconds should be used in this case, (11.5 r.p.m) in order to get flakes with good storage stability.

### ***Textural Characteristics of Reconstituted Cassava Flakes Mash***

It is desirable that reconstituted cassava flakes mash have the same characteristics as the mash from cooked fresh cassava roots. However, this was not the case. On reconstitution cassava flakes gave a very sticky mash. Different types of emulsifiers were added to find out which one was suitable for improving the textural characteristics of the reconstituted cassava flakes mash. Skim milk powder and sodium caseinate did not reduce the stickiness of the reconstituted cassava flakes mash. Samples with stearate monoglyceride were less sticky indicating that this was the most suitable emulsifier to be used. Stickiness results obtained by the TPA test were similar to those obtained by the Back extrusion test.

Formation of amylose-monoglyceride complexes helps to remove free starch from the medium during flakes production (Hoover and Hadziyev, 1981; Szczodrak and Pomeranz, 1992; Kweon *et al*, 1994). The fact that reconstituted cassava flakes mash with stearate monoglyceride in this study was less sticky, indicates the formation of an amylose-monoglyceride complex.

This was further confirmed by the DSC measurements, in which the thermogram showed a peak between 119-162°C for samples which contained stearate monoglyceride and none for the rest of the samples with other emulsifiers. The Brabender amylographs also indicated that samples with stearate monoglyceride had low viscosity, which shows that the formation of amylose-monoglyceride complexes inhibit excessive swelling of starch granules which is responsible for high viscosity in heated starchy foods.

The results also showed that cassava flour samples with stearate monoglyceride had lower water absorption capacity (WAC) compared to samples without emulsifier and those with skim milk powder and sodium caseinate. At the same time, the bulk density of cassava flour with stearate monoglyceride was higher than that of samples without emulsifier and those with skim milk powder and sodium caseinate.

The results of the microscopy also showed that starch granules of gelatinised cassava flour samples and flakes with stearate monoglyceride did not burst completely during heating and drying. Starch granules of samples without emulsifier and those with skim milk powder and sodium caseinate disintegrated completely. So, stearate monoglyceride, as observed earlier, prevented starch granules from excessive swelling, thus preventing the granule from bursting completely. It was also observed that reconstituted cassava flakes mash with stearate monoglyceride were whiter than the rest of the samples. This is a good attribute especially for consumer acceptability.

Another measure for reducing the pastiness of reconstituted cassava flakes mash is by allowing the gelatinised starch to retrograde by adding the pre-cooking and cooling stages in the production of the flakes. The results showed that pre-cooked and cooled samples had lower free soluble amylose than samples which were not pre-cooked. Low free amylose content was observed after the cooling stage. So the association of free amylose reduces the stickiness of cassava samples (Jankowski, 1992).

When sensory and instrumental methods were compared in measuring the textural characteristics and colour of reconstituted cassava flakes mash, the results showed good correlation. This indicates that either method can be used to measure different attributes of cassava flakes, especially the textural characteristics.

## ***Main Conclusions***

- Wet fermentation is very effective in reducing cyanogens of cassava to safe levels than solid-state fermentation.
- Solid-state fermentation increases the protein content of cassava and retains higher levels of vitamin C compared to wet fermentation.
- Samples processed by sun drying retain higher vitamin C content but contain higher residual cyanogens.
- Mould growth on cassava during solid-state fermentation does not always result in production of aflatoxins.
- Pre-cooking of cassava (during the production of the flakes) results in an increase of moisture content and a decrease of free starch and vitamin C content of the flakes.
- Stearate monoglyceride reduces the stickiness of reconstituted cassava flakes mash.
- The colour of reconstituted cassava flakes mash samples with stearate monoglyceride was whiter than samples with other emulsifiers and fresh cassava.

## ***Suggestions for Further Work***

This research work has successfully used the drum drier to produce cassava flakes. There are however, still some gaps to be filled before the cassava flakes can appear on the shelves. In view of this, the following are my suggestions for further work to be done on cassava flakes production:

- To develop a combination of treatments which would eliminate cyanogens, thereby allowing for the production of edible flakes from the bitter cassava varieties.
- To carry out further sensory tests for assessing the organoleptic characteristics of plain and flavoured cassava flakes.

- To optimise the drum drying process using Response Surface Methodology in order to obtain the best colour and flavour .
- To determine the moisture sorption characteristics of the cassava flakes and relate these to packaging requirements and shelf life.
- To investigate the role of anti-oxidants or fortification agents as a means of retaining the nutritional components such as vitamin C.
- To evaluate the cost benefit of producing cassava flakes by drum drying in a developing country.

## Chapter 8

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## Appendix 1

### VARIABLES FOR CALCULATING TOTAL, INTERMEDIATE + FREE CYANOGENS

Solid state fermentation. Tanga.

Village1

Sample1

	V (ml)	V'	Eq.A620	A620	DW (g)	H2O%	CN(mg/ kg)	Av. CN
Total cn	180	184.86	0.815	0.166	25.12	16.21	15	14.5
	180	184.86	0.815	0.154	25.12	16.21	13.9	
Int.&Fcn	180	184.86	0.778	0.015	25.12	16.21	1.4	1.6
	180	184.86	0.778	0.019	25.12	16.21	1.8	
Fcn	180	184.86	0.774	0.009	25.12	16.21	0.9	1.0
	180	184.86	0.774	0.012	25.12	16.21	1.1	

Sample2

Total cn	180	184.22	0.815	0.219	25.78	14.07	19.2	17.9
	180	184.22	0.815	0.188	25.78	14.07	16.5	
Int.&Fcn	180	184.22	0.778	0.033	25.78	14.07	3.0	2.7
	180	184.22	0.778	0.025	25.78	14.07	2.3	
Fcn	180	184.22	0.774	0.019	25.78	14.07	1.8	1.6
	180	184.22	0.774	0.015	25.78	14.07	1.4	

Sample3

Total cn	180	184.02	0.815	0.131	25.98	13.40	11.4	12.5
	180	184.02	0.815	0.156	25.98	13.40	13.6	
Int.&Fcn	180	184.02	0.778	0.019	25.98	13.40	1.7	1.9
	180	184.02	0.778	0.023	25.98	13.40	2.1	
Fcn	180	184.02	0.774	0.012	25.98	13.40	1.1	1.2

## Appendix 2

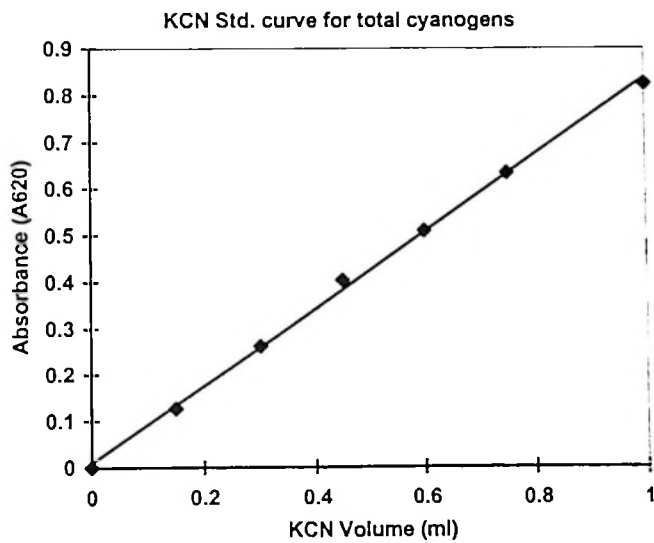
### KCN STANDARD CURVES

KCN Standard Curves (Cyanide Assay)

Total Cyanide Simulation (ml)

	S <sub>0</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	S <sub>6</sub>
pH 7.0	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Extraction Medium	0.1	0.1	0.1	0.1	0.1	0.1	0.1
0.2M N <sub>a</sub> OH	0.6	0.6	0.6	0.6	0.6	0.6	0.6
pH 6.0	2.90	2.75	2.60	2.45	2.30	2.15	1.90
KCN / P	0.00	0.15	0.30	0.45	0.60	0.75	1.00
A <sub>620</sub>	0.000	0.127	0.261	0.403	0.509	0.632	0.823

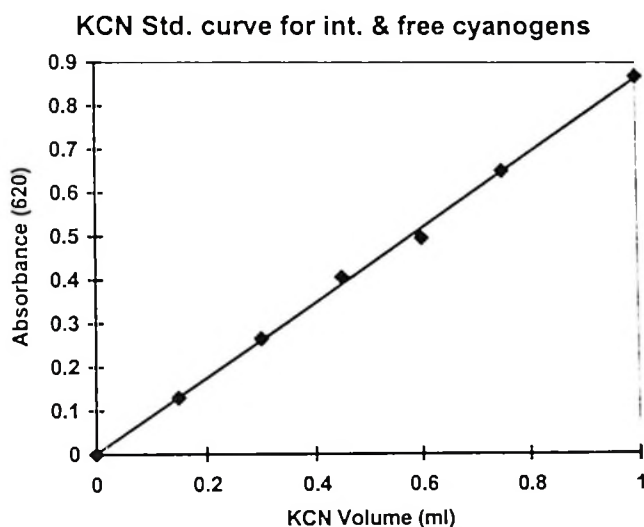
Then follows colorimetry as usual for all the tubes.



Intermediate & Free Cyanide Simulation (ml)

	S <sub>0</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	S <sub>6</sub>
pH 4.0	3.30	3.15	3.00	2.85	2.70	2.55	2.30
Extraction Medium	0.1	0.1	0.1	0.1	0.1	0.1	0.1
0.2 M. N <sub>a</sub> OH	0.6	0.6	0.6	0.6	0.6	0.6	0.6
KCN / P	0.00	0.15	0.30	0.45	0.60	0.75	1.00
A <sub>620</sub>	0.000	0.129	0.264	0.406	0.495	0.650	0.867

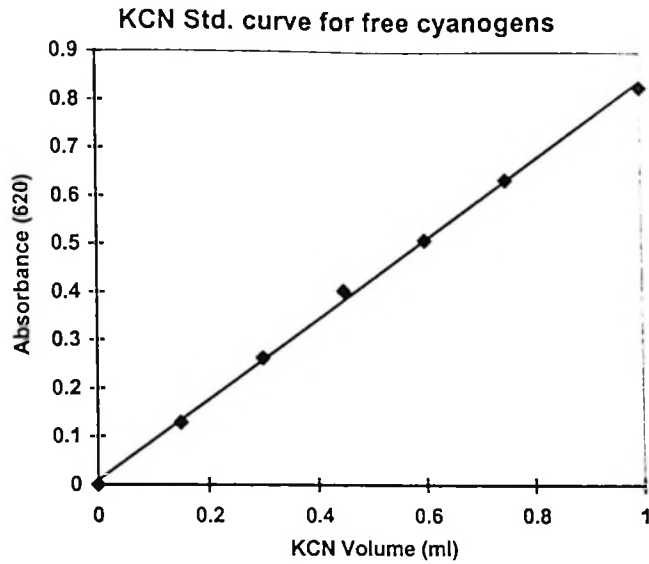
Then follows colorimetry as usual for all the tubes.



Free Cyanide Simulation (ml)

	S <sub>0</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	S <sub>6</sub>
pH 4.0	3.90	3.75	3.60	3.45	3.30	3.15	2.90
Extraction Medium	0.1	0.1	0.1	0.1	0.1	0.1	0.1
KCN / P	0.00	0.15	0.30	0.45	0.60	0.75	1.00
A <sub>620</sub>	0.000	0.129	0.263	0.402	0.508	0.634	0.828

Then follows colorimetry as usual for all the tubes.



Example of calculations.

0.1242 g KCN dissolved in 500 ml of 0.2 M sodium hydroxide

Therefore, = 0.2484 g/l KCN

Dilution factor 100.

= 0.002484 g/l KCN

= 2.484 mg/l KCN

= 2.484  $\mu$ g/ml KCN

m.wt. of HCN = 27

m.wt. of KCN = 65

Therefore,  $2.484 \times \frac{27}{65} = 1.03 \mu\text{g/ml HCN}$

1 ml KCN/P ----- 1.03  $\mu$ g HCN

Taking S<sub>4</sub> 0.6 ml of KCN added.

This has 0.618  $\mu$ g HCN = A - 508

$$\text{Therefore, } 1\mu\text{g HCN} = A = \frac{0.508}{0.618} = 0.822$$

(correction factor for purity of KCN - 97%)

$$A = \frac{0.822}{0.97} = 0.847$$

$$\text{Free Cyanide Equiv. } A_{620} = \underline{0.847}$$

## **Methodology for cyanogens determination**

### **Cyanogens Extraction**

Cassava flour, 30 g of sample was used (in triplicate). The extracting medium used was 0.1 M orthophosphoric acid with 25% v/v ethanol. The samples were blended in a Waring blender with 160 ml of extracting medium for two minutes, with an interval of one minute. Filtration was done by using glass fibre (GF/A paper). Then the blender was washed by 20 ml of extracting medium.

### **Standard Curves**

Each time the enzymatic assay was done, fresh standards were prepared, concurrent with the assay of the samples. Standards were made as follows : dried potassium cyanide, 125 mg were dissolved in 500 ml of 0.2 M sodium hydroxide, which was then diluted 100 times in 0.1 M phosphate buffer, pH 6.0 to give a solution of 2.5  $\mu\text{g/ml}$  KCN. (Details and Curves are in Appendix 2). These were used to calculate the absorbance which corresponds to 1 $\mu\text{g}$  of cyanogens (Equivalent  $A_{620}$  1 $\mu\text{g}$  HCN) required in the formula for cyanogens content calculations.

## **Cyanogens Determination by Enzymatic Assay**

By this method, free cyanide (HCN), intermediate plus free cyanide and total cyanogens (free cyanide + intermediate cyanide + cyanogenic glucosides) were determined. For free cyanide (HCN), 3.9 ml of pH 4.0 phosphate buffer were added into a test tube, followed by 0.1 ml of extract. Then, 0.2 ml of chloramine T. The tubes were kept in an ice/water bath for five minutes then pyridine reagent added. At this stage the colour development began (pink to blue) and tubes were kept in a fume cupboard for 90 minutes, then spectrophotometer readings taken at 620 nm. For the intermediate plus free cyanide determination, 0.1 ml extract was added to 0.4 ml pH 4.0 phosphate buffer in the test tube. This was followed by 0.6 ml of sodium hydroxide (0.2 M), then 2.9 ml of pH 4.0 phosphate buffer were added. Next the colorimetric procedure was followed as in the free cyanide assay.

Total cyanogens were also determined in the same way, 0.4 ml of pH 7.0 phosphate buffer being added into test tubes followed by 0.1 ml of an extract. In this case linamarase enzyme was added, (0.1 ml) and incubated in a water bath at 30°C for 15 minutes. (The enzyme was added at an interval of 20 seconds). Then 0.6 ml of sodium hydroxide (0.2 M) was added (at the interval of 20 seconds exactly, to make sure all the tubes had the same incubation time of 15 minutes). Next, 2.8 ml of pH 6.0 phosphate buffer was added followed by the colorimetric procedure as in the assays for free and intermediate plus free cyanide. The blanks in all three assays were prepared in the same way as samples, except that 0.1 ml of extracting medium was used instead of extract.

## Calculation of Cyanogens Content in Extracts

According to Cooke (1979) the cyanogens content of an extract of cassava is derived as follows:

$$\frac{10V \times A}{\text{Equivalent } A_{620} \text{ of } 1 \mu\text{g HCN}} \times X \quad \mu\text{g HCN/g cassava}$$

where:

V = volume of extract obtained after filtration/ centrifugation (ml)

A = mean  $A_{620}$  from extract

X = weight (dry) of sample from which extract was made (g).

More recently this equation has undergone some modifications. Firstly, as a result of investigations at ODNRI, involving the incorporation of exogenous acetone cyanohydrin in Nigerian gari (a fermented, cooked, cassava food), it is indicated that, the cyanogens content of the recovered extract is equal to that of the portion retained by the cassava homogenate. Secondly, it is thought that the moisture from the cassava sample itself will dilute the extract during sample homogenisation. In that case the moisture content of the sample should be taken into account, when considering the volume of extract. These adjustments give rise to a new equation which is :

$$\frac{10V^1 \times A}{(\text{Equivalent } A_{620} \text{ of } 1 \mu\text{g HCN}) \times Xd} \quad \mu\text{g HCN /g cassava}$$

where:

A = mean  $A_{620}$  from extract

Xd = dry weight of sample from which extract made (g)

$V^1$  = adjusted volume as : 
$$V^1 = \frac{V + (Xf \times \% H_2O)}{100}$$

where :

V = volume of extraction medium used (ml)

Xf = fresh weight of sample from which extract made (g).

### **Extraction of Aflatoxins**

Cassava flour sample (50 g) was placed in 500 ml glass-stoppered Erlenmeyer and 200 ml of methanol-water (85 + 15) added and the stopper secured with a masking tape. The samples was shaken vigorously by hand then on a wrist-action shaker for 30 min. Then the mixture was filtered through a medium fluted paper. The filtrate (40 ml) was collected into 125 ml separating funnel, to which 40 ml of sodium chloride were added. After shaking for 1 minute, phases were allowed to separate and the lower (aqueous) phase drained into another 125 ml separating funnel, while the upper phase was discarded. Aflatoxins were extracted from the aqueous phase with two 25 ml portions of methylene chloride, by shaking for 1 minute. The fractions were combined into 125 ml Erlenmeyer flask and evaporated to dryness on steam bath.

### **Thin Layer Chromatography**

Samples were dissolved in 250  $\mu$ l  $CH_2Cl_2$ , and spotted on the TLC plates. Aflatoxin B<sub>1</sub> standard 5, 10, 15 and 20  $\mu$ l also were spotted alongside samples. The plates were

developed with methylene chloride -acetone (7 + 3) for about 40 min. and evaporated in a fume cupboard for 5 min. followed by 2 min. in a 50°C forced draft oven.

Plates were examined under long-wave UV light to determine the presence or absence of aflatoxins. The TLC detection limit is 1 ng (Lovelace *et al*, 1989). Aflatoxin B<sub>1</sub> standard solutions were prepared in toluene-acetonitrile (98 + 2), calculated to give concentration of 8-10 µg/ml. The vortex shaker was used.

In order to calculate the true quantity of aflatoxins, confirmation methods need to be performed (Wheatley and Cock, 1985; Cooke and Tomlins, 1986; Wareing and Westby, 1993). The confirmation can be done by spraying the TLC plate with 50% sulphuric acid. Aflatoxins produce a characteristic yellow colour whereas scopoletin does not change. Further confirmation is achieved by two-dimensional co-chromatography. Two 2-D plates should be developed firstly in chloroform:acetone (9:1) and secondly in diethyl ether:ethanol:water (94:4.5:1.5), where the plate is 'spiked' before development with a mixture of aflatoxins (Cooke and Tomlins, 1986).

#### **Free starch determination**

The free starch content of cassava flakes was determined by the AOAC method (AOAC, 1984). A mixture of flour sample (2.0-2.5 g), water, (10 ml) and CaCl<sub>2</sub> containing 0.8% HOAc (60 ml) was boiled with frequent stirring for 15 min. The solution was cooled rapidly and filtered (Whatman 42 or 44). Starch in the filtrate was determined polarimetrically .

$$\% \text{ Starch} = 100 \times R \times 100/1 \times 203 \times W = 49 \times R/W$$

Where  $R$  is observed angular rotation,  $l$  is length of tube and  $W$  is weight of sample, 203 is arbitrarily taken as the specific rotation for all starches.

**Soluble amylose according to Wotton *et al* (1971) method.**

The soluble amylose was determined as follows: Cassava samples (2 g) were macerated with water (100 ml) in a waring blender for 1 min. The suspension was centrifuged at  $2000 \times g$  for 10 min. at  $20^{\circ}\text{C}$ . Duplicate aliquots (1 ml) were diluted to 10 ml with water and iodine solution (4% KI, 1%  $\text{I}_2$ ; 0.1 ml) added. Absorbance ( $A_1$ ) at 600 nm in 10 mm cell was then measured against a reagent blank. A further suspension of (2 g) in water (95 ml) was prepared as described above. To this suspension was added potassium hydroxide solution (10 M, 5 ml) and the mixture was allowed to stand for 5 min. with gentle agitation. The alkaline suspension was then centrifuged. Duplicate aliquots (1 ml) were then mixed with hydrochloric acid (0.5 M, 1 ml) and diluted up to 10 ml with water. Iodine solution (0.1 ml) was then added and absorbance ( $A_2$ ) at 600 nm determined as described above. Extent of gelatinisation was calculated as  $(A_1/A_2 \times 100)\%$ .

**Cassava starch was extracted according to Moorthy *et al* (1996) method.**

Starch was extracted from chopped cassava roots using a waring blender, passing the slurry through a  $55 \mu\text{m}$  sieve and allowing the starch to sediment overnight. Settled starch was dried in an oven at  $40^{\circ}\text{C}$  for 72 hours, turning occasionally at the first stages of drying. Emulsifiers were added to cassava starch and mash (2%). In the case of starch some water was added to form a suspension. Cassava starch suspensions and cassava mash were dried in an air draft assisted oven at  $50^{\circ}\text{C}$  for 8 hours to allow starch-

emulsifier complex formation. Water was added to dried cassava starch samples containing emulsifiers to form suspensions (1:3 starch -water ratio). The DSC measurements were made on a Perkin Elmer DSC7. Indium was used to calibrate the instrument.

**The water absorption characteristics (WAC) according to Onuma and Bello (1988) method**

One gram of each sample in a conical graduated centrifuge tube was thoroughly mixed with 50 ml distilled water at high speed in a Waring -whirl mixer (Hooke and Tucker, Ltd). The samples were then allowed to stand at room temperature for 30 min. and centrifuged at 5000 x g for 30 min. The volume of free water was read directly from the graduated tube. The amount of water absorbed was calculated as total water minus free water.

### Appendix 3

## STATISTICAL ANALYSIS OF CYANOGENS IN CASSAVA SAMPLES

Variance components analysis was carried out in which three model were compared:

#### Model 1

Allowing for variation between regions, villages (within regions) and samples (within villages).

#### Model 2

Allowing for variation between regions, and samples (within regions).

#### Model 3

Allowing for variation between sample only.

The difference in the statistic, -2REML Log Likelihood, between model 1 and model 2 was compared to  $\chi^2$  with 3 degrees o freedom and similarly for model 3 and 1 with 71 degrees of freedom.

PROPORTION OF SAMPLES WITH TOTAL CYANOGENS GREATER THAN 10 mg HCN.kg<sup>-1</sup>.

Region 1. Solid -State fermentation Tanga region.

Total cyanogens, Mean =13.89, Variance=1.94.

$$\begin{aligned} &N(13.89, 1.94^2) \\ &Pr(X>10) \\ &=1-\Phi\left(\frac{10-13.89}{1.94}\right) \\ &=1-\Phi(-2.005) \\ &=1-1.022 \\ &=0.978 \end{aligned}$$

The probability of samples having total cyanogens levels greater than 10 mg HCN.kg<sup>-1</sup> is 97.8%.

Region 2. Solid -State fermentation Mwanza region.

Total cyanogens, Mean =13.95, Variance=1.904.

The probability of samples having total cyanogens levels greater than 10 mg HCN.kg<sup>-1</sup> is 98.1%.

Region 3. Wet fermentation Tanga region.

Total cyanogens, Mean =5.867, Variance=2.668.

The probability of samples having total cyanogens levels greater than 10 mg HCN.kg<sup>-1</sup> is 6.1%.

Region 4. Sun drying Kagera region.

Total cyanogens, Mean =6.800, Variance=2.396.

The probability of samples having total cyanogens levels greater than 10 mg HCN.kg<sup>-1</sup> is 9.0%.

## Appendix 4

### ANOVA TABLES FOR CASSAVA FLAKES

Anova table for Moisture.

Source	DF	SS	MS	F	Prob.
DRUM	1	25.134	25.134	244.2	0.000
HEAT	2	33.05	16.525	160.6	0.000
DRUMHEAT	2	2.9209	1.4605	14.2	0.001
Error	12	1.2351	0.10293		
Total	17	62.34			

Anova table for Vitamin C.

Source	DF	SS	MS	F	Prob.
DRUM	1	0.24036	0.24036	7.5	0.018
HEAT	2	23.764	11.882	370.6	0.000
DRUMHEAT	2	1.7954	0.89769	28.0	0.000
Error	12	0.38473	0.03206		
Total	17	26.185			

Anova table for Reducing sugars.

Source	DF	SS	MS	F	Prob.
DRUM	Ommited-ssq	almost zero			
HEAT	2	0.40373	0.20187	41.7	0.000
DRUMHEAT	2	0.51893	0.25947	53.6	0.000
Error	13	0.06293	0.00484		
Total	17	0.9856			

Anova table for Protein.

Source	DF	SS	MS	F	Prob.
DRUM	1	0.00001	0.00001	0.2	0.690
HEAT	2	0.00003	0.00002	0.5	0.619
DRUMHEAT	2	0.00001	0.00001	0.2	0.848
Error	12	0.0004	0.00003		
Total	17	0.0045			

Anova table for starch.

Source	DF	SS	MS	F	Prob.
DRUM	1	0.00467	0.00467	0.0	0.840
HEAT	2	371.71	185.85	1698.3	0.000
DRUMHEAT	2	0.46081	0.23041	2.1	0.165
Error	12	1.3132	0.10943		
Total	17	373.49			

Anova table for Adhesiveness.

Source	DF	SS	MS	F	Prob.
EMULS	3	5416215	1805405	3354.1	0.000
Error	12	6459.3	538.27		
Total	15	5422674			

Anova table for Extrusion.

Source	DF	SS	MS	F	Prob.
EMULS	3	268045676	89348559	535.2	0.000
Error	12	2003459	166955		
Total	15	270049135			

## Appendix 5

### INSTRUCTIONS FOR PANELLISTS AND THE INTENSITY SCALES

Name:  
Course:  
Date:

You will receive 3 samples of cooked cassava. The first sample labeled 'R' is the **reference sample**.

1. Examine and score the colour of the samples.
2. Remove a small portion of the product with your fingers, manipulate it in the hand and evaluate for:
  - Firmness
  - Stickiness/Adhesiveness

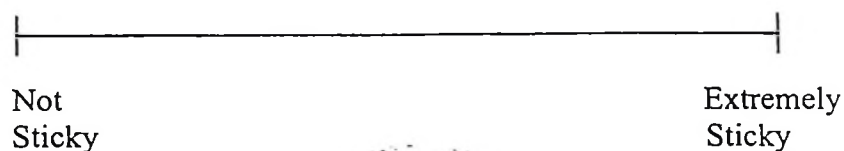
1. Colour.



2. Firmness.  
Resistance to compression between thumb and forefinger.



3. Stickiness/Adhesiveness.  
Easiness of shaping into the ball and degree of coating of palms.



8/2/99

S/P  
AS 9025  
C30  
M8