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DECEMBER 2005

MASTER OF PHILOSOPHY

LUCAS DANIEL TIVANA

Title of Thesis

"A STUDY OF HEAP FERMENTATION AND PROTEIN ENRICHMENT OF CASSAVA"

Subject to Approval by Council)

N. TAKAWIRA Ntakawira DATE 15.12.05
DEPUTY REGISTRAR (ACADEMIC)

A STUDY OF HEAP FERMENTATION AND PROTEIN ENRICHMENT OF CASSAVA

by

LUCAS DANIEL TIVANA

A thesis submitted in partial fulfilment of the requirements for the degree of Master of Philosophy

Institute of Food, Nutrition and Family Sciences
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University of Zimbabwe
January 2005

ABSTRACT

Cassava (*Manihot esculenta* Crantz) is an important source of carbohydrate for humans and animals, producing high energy of 610 kJ/100 g fresh root weight. However, it is very poor in protein (1 % fresh root weight) and contains cyanogenic glucosides which are related to various health disorders that occur in populations where cassava is the staple food. Cassava products that are not adequately processed have been linked to diseases like *konzo*, caused by cyanide poisoning, as was the case in Nampula Province, Mozambique.

Samples of cassava flour were collected in 4 different districts in Nampula Province, Mozambique and the cassava processing methods were recorded. Cassava processing techniques used in Nampula Province consist of peeling, chipping or grating, sun-drying or fermenting followed by sun-drying, and finally pounding into flour. There was a large variation in the average cyanogenic potentials of flours from the different districts, ranging from 26 $\pm$ 20 to 90 $\pm$ 60 mg HCN/kg. The average total cyanogenic content for the unfermented samples (64 $\pm$ 60 mg HCN/kg) was significantly greater than that of the fermented samples (34 $\pm$ 30 mg HCN/kg).

Biochemical and microbial changes occurring during the heap fermentation of cassava roots were determined and predominant micro-organisms were isolated and identified. The total crude protein and cyanogenic potential were determined in dried fermented and unfermented cassava flour. The moulds, *Rhizopus stolonifer* and *Neurospora sitophila* were the dominant microbes involved in the heap fermentation of cassava followed by lactic acid bacteria, *Leuconostoc pseudomesenteroides*, *Leuconostoc mesenteroides*, *Enterococcus faecium* and *Weissella cibaria*. The pH values of the cassava roots decreased from 6.1 $\pm$ 0.01 to 5.6 $\pm$ 0.6 during heap fermentation.

Heap fermentation of cassava resulted in a decrease in the total cyanogenic potential levels. The average total cyanogenic level in unfermented cassava flour was 158 mg HCN/kg, while in fermented cassava flour, a value of 17 mg HCN/kg was recorded. The average cyanogenic potential of fresh cassava roots was 259 $\pm$ 9 mg HCN/kg. Protein concentration in the cassava flour slightly increased from 1.3% to 1.8% w/w dry matter during fermentation.

Laboratory simulation of the heap fermentation of cassava roots using isolated moulds was carried out to determine the growth and change in texture of the cassava roots. *Neurospora sitophila* grew faster than *Rhizopus stolonifer* on cassava roots under controlled conditions. *Rhizopus stolonifer* softened the cassava roots more than the *Neurospora sitophila*. Slicing the cassava roots increased the rate of mould growth and the softness of the roots during the fermentation.

Studies were carried out to increase the protein content of cassava flour by the co-fermentation of cassava roots with cowpea (*Vigna unguiculata*) using selected moulds. Co-fermentation of cassava roots with cowpea flour, at a proportion of 92:8 (cassava:cowpea), resulted in faster growth of moulds, rapid softening of cassava roots and an increase in the protein content of the flour. The final protein content in cowpea supplemented cassava flour was 7.93 $\pm$ 0.98 % dry weight basis, similar to the maize grain. The flour produced from cassava roots co-fermented with cowpea produced a paste (*karakata*) of lower viscosity and higher sensory acceptability compared to that prepared using flour from cassava roots fermented without cowpea.

Heap fermentation of cassava roots reduced the cyanogenic potential of the roots but did not achieve the FAO/WHO recommended safe limit of 10 mg HCN/kg when bitter varieties were used. Supplementation of cassava roots with cowpea produced a flour with lower cyanogen content, higher protein content and lower viscosity compared to flour produced from unsupplemented cassava roots.

ACKNOWLEDGEMENTS

I would like to express my personal thanks to:

My supervisor, Dr. Jane Bvochora, for her valuable scientific discussions, encouragement and support to take the research to the end.

My previous supervisors, Professor Remigio Zvauya and Dr. Anthony Mutukumira for their constructive contribution to the research project.

Dr José da Cruz Francisco of Eduardo Mondlane University, Mozambique, for introducing me to field of cassava research.

Dr. John David Owens of the School of Food Bioscience, The University of Reading, for the numerous ideas, constructive discussions, for allowing me to work in his laboratory, assistance and support in the area of lactic acid bacteria and mould identification and laboratory simulation of cassava roots fermentation using isolated moulds.

Professor Louis Mutombene Pelembe of Eduardo Mondlane University, Mozambique for assistance in sensory evaluation of cassava flour prepared from the laboratory.

Dr Maria Isabel Andrade of South Africa Root crop network (SARNET)-Mozambique, eng Anabela Zacarias of Instituto Nacional de Investigação Agronómica, eng Paulo Mabote and Abudo Jone of World Vision-Mozambique for providing material, transport and assistance in fieldwork.

Dr J. Howard Bradbury of Australian National University for his assistance and support in the area of cyanogen determination.

Professor Daniel Tevera of University of Zimbabwe, Mrs Tevera and Mrs Margaret Chimbira for providing living facilities during my stay in the Republic of Zimbabwe.

The following people who played important roles at various stages of the project: Mario Ernesto, Paula Cardoso, Dr Tapiwa Mugochi, Talent Bvochora, Dr Carlos Lucas, Dr Luís Amós, Vitória Nhanombe, Jorge Nhantitima, Abisha Kasiyamhuru, eng Maida Khan, Alfred Bere, Kudakwashe Chitindingu and Fungayi Chatiza.

The Eduardo Mondlane University (UEM) and Swedish Agency for Research Cooperation with developing Countries (SAREC) for financial support of the research.

To my wife, eng Anabela Casimiro Chambule and my two daughters, Júlia and Sara Tivana for their patience and moral support.

This MPhil thesis is based on the following papers:

1. Zvauya R., Ernesto M., Bvochora T., Tivana L., Francisco J., (2002). A study of the effect of village processing methods on the cyanogenic potential of cassava flours collected from selected districts in Nampula Province, Mozambique. *International Journal of Food Science & Technology* , **37**, 463-469.
2. Tivana L. D., Bvochora J., Owens J. D. & Mutukumira A. N. (2003). "A study of heap fermentation of cassava (*Manihot Esculenta* Crantz) in Nampula Province, Mozambique". *Proceedings of 13th Triennial ISTRC Symposium, 9-15 November 2003*, Arusha Tanzania. In press
3. Tivana, L. D., Bvochora, J. M, Owens J. D. & Mutukumira A. N. Co-fermentation of cassava roots with cowpea using isolated moulds for protein enrichment of cassava flour. Manuscript
4. Tivana, L. D., Bvochora, J. M. & Pelembe, L. M. Sensory evaluation of cassava flour paste (*karakata*) prepared using flour from fermented cassava roots supplemented with cowpea. Manuscript

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1.0 INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a dicotyledonous plant, belonging to the family Euphorbiaceae (Alves, 2002). It is a perennial shrub, 2 to 4 m in height and is mainly propagated from stem cuttings. Cassava forms a staple food for an estimated 500 million people in the tropics. It is widely grown in most countries in the tropical regions of Africa, Latin America and Asia. Cassava is grown over a range of climates and altitudes and on a wide variety of soils. Cassava is tolerant to drought and is productive in poor soil where other staple crops cannot grow (Bradbury and Holloway, 1988). The crop is an important source of carbohydrate for humans and animals, having higher energy than other root crops, 610 kJ/100 g fresh weight. Dried cassava root has energy similar to the cereals (Bradbury and Holloway, 1988, FAO, 1990). In Africa, the continent with the largest cassava production, about 93% of the produce is used as food (Nweke *et al.*, 2002).

Mozambique, which produced 6.15 million tons of cassava in 2003, ranks 5th in cassava production in Africa (Sistema Nacional de Aviso Prévio, 2003). In Mozambique, cassava is the second most important staple food after maize (INIA & SARRNET, 2003) and contributes 42% of the total energy intake (Dahniya, 1994).

Nampula province, Mozambique, with approximately 3 million people, about 20% of the population of Mozambique, is the largest producer of cassava in Mozambique, with an average of 2.22 million tons per year and an average yield of 5.5 tons per hectare (Sistema Nacional de Aviso Prévio, 2003). Cassava forms the staple food of the people in this province. The rural people usually eat a thick porridge known as *karakata* made from cassava flour and served with beans for most meals. Cassava grown in this region consists mostly of bitter varieties, containing high cyanogen levels. These bitter varieties are, however, popular because they give high yields and are resistant to disease and attack by pests and animals such as

monkeys, which eat the sweet varieties. It has been suggested that the bitter varieties were introduced during the Mozambican war, 1975-1992, as the peasants were not able to go into the fields to guard their crops from animal predation. Consumption of cassava products that are not adequately processed has been linked to diseases like *konzo* (epidemic spastic paraparesis), which are caused by prolonged cyanide exposures, as was the case in Nampula Province during periods of drought and war (Casadei *et al.*, 1990). Sporadic cases of *konzo* continue to be reported in the province, with 27 new cases having been observed during the period 1997-1999 (Ernesto *et al.*, 2002). Such health disorders are associated with under-nourishment, especially deficiency in protein (Delange *et al.*, 1994; Teles, 1995).

The cassava root processing techniques used in Nampula Province, Mozambique, consist of peeling, chipping or grating, fermenting, sun-drying and finally pounding into flour. A combination of these techniques is used in most villages. Because of the high levels of cyanogens in the bitter varieties, the processing techniques used are important in producing a safe product. Despite the widespread consumption of bitter varieties in Nampula Province, very few studies have been done on the cyanogenic potentials of cassava products from rural homes and were limited to two districts (Essers *et al.*, 1996; Cardoso *et al.*, 1998; Ernesto *et al.*, 2000). There is a need, therefore, to study the influence of cassava processing techniques on the cyanogenic potentials of cassava so as to recommend techniques, which lower the cyanogenic potential levels. The most widely used cassava root processing technique used in Nampula province for reducing cyanogens is heap fermentation, which has not been studied in detail previously in this province. The heap fermentation process was studied in Uganda by Essers *et al.* (1995a).

Cassava roots are very poor in protein (about 1% fresh weight). The proteins in cassava contain a low percentage of essential amino acids, such as lysine, methionine and tryptophan (FAO, 1990; Teles, 1995). Leucine, phenylalanine and threonine are also low in cassava

compared to other tuber crops (Ciacco & Appolonia, 1977; FAO, 1990). Cassava protein is 50% utilizable by the body (FAO, 1990). Previous studies have shown that heap fermentation slightly increases the protein concentration in cassava roots (Essers *et al.*, 1995a).

Fresh or processed cassava is usually combined, in meals, with other foods such as meat, fish and vegetables. During drought times in Mozambique, usually in Nampula Province, the only available foodstuff is cassava and the population of this region presents many malnutrition cases. The available cassava usually possesses high cyanide content and during these times people usually process the cassava roots inadequately.

One of the aims of this study was to relate the cassava processing methods to the cyanide levels of flours collected from different districts in Nampula Province, Northern Mozambique. The study also aimed at determining biochemical and microbial changes occurring during heap fermentation of cassava roots. Efforts were directed at increasing the protein content of cassava flour by a combination of solid-state fermentation using selected moulds and supplementation of cassava roots with cowpea (*Vigna unguiculata*). The influence of cowpea supplementation on mould growth, texture of cassava roots and viscosity of the cassava flour was investigated.

The study also aimed at determining the consumer acceptability of *karakata* prepared using flour from cassava roots, either supplemented with cowpea or not and fermented with either *Rhizopus stolonifer* or *Neurospora sitophila*. Cyanogenic potentials and sensory properties of the fermented cassava flours prepared under the various fermentation conditions were investigated

2.0 LITERATURE REVIEW

2.1 ORIGIN AND BOTANY OF CASSAVA

2.1.1 The origin and distribution of cassava

Cassava has its genetic, geographical and agricultural origin in Latin America. Its domestication began 5000 – 7000 years BC in the Amazon, Brazil (Allen, 2002) and it was distributed by Europeans to the rest of the world (Henry & Hershey, 2002). Cassava was taken from Brazil to the West coast of Africa by Portuguese navigators in the 16th century (Jones, 1959, Nweke, 1994). Cassava was brought to East Africa in the 18th century by the Portuguese from Cape Verde and into Mozambique from Zanzibar Island (Leitão, 1970). It was introduced to most of Asia and the Pacific in the late 18th and early 19th centuries (Onwueme, 2002).

2.1.2 Taxonomy of cassava

Cassava, as known in English, is "manioc" in French , "yuca" in Spanish, and "mandioca" in Portuguese. Cassava belongs to the class Dicotyledoneae, family Euphorbiaceae, tribe *Manihoteae*, genera *Manihot* Tournefort and species *Manihot esculenta* Crantz (Alves, 2002).

2.1.3 Physiology and morphology of cassava

Cassava is a perennial shrub, 2 to 4 m in height. It is mainly propagated from stem cuttings. Propagation by seed results in genetically diverse plants and is used for generation of new varieties. Cassava can also be propagated by "mini-stem" for rapid propagation and by tissue culture to propagate plants free of pathologies (Rulkens, unpublished).

Cassava propagated by the stem can grow indefinitely, alternating periods of vegetative growth, storage of carbohydrates in the roots, and even periods of almost dormancy. The dormancy of the cassava plant occurs when there are severe climatic conditions, such as low temperature or prolonged water deficit (Alves, 2002). On average, cassava storage roots are ready for harvest from 12 months after planting, except for precocious varieties that can be harvested before 12 months. The time until harvest is also dependent on environmental conditions.

The cassava plant is characterized by the following parts:

Stem- The stem is 1 to 4 m long and woody with a thick bark. The old part of the stem bears evident scars of fallen first leaves. The system of stem branching is controlled by genetic and environmental factors (CIAT, 1981; IITA, 1990). The branching may start at any time of plant growth, producing 3 new branches and after a certain time these produce 3 more new branches each. The level of branching depends on the variety of cassava (Rulkens, unpublished).

Leaves- Cassava leaves have a long petiole and are divided into 5 to 7 lobes. The leaves have a spiral insertion on the cassava stem with a phyllotaxy of 2/5. New leaf and petiole colour depend on the genotype.

Inflorescences- Cassava is a monoecious species with panicle inflorescences at the reproductive branching points, female flowers (staminate) in the base and male flowers (pistillate) at the top of the inflorescence (Alves, 2002).

Fruit- the fruit is a result of cross-pollination. It is a globular capsule, trilocular, 1 to 1.5 cm in diameter with six straight longitudinal aristae (CIAT, 1981). Each locule contains a single carunculate seed (Alves, 2002).

Roots- roots are the main storage and the most important organ in cassava for humans. The cassava root is not a tuberous root, but a true root which cannot be used for vegetative

propagation (Alves, 2002). The mature cassava storage root has four distinct tissues (Figure 2.1): bark (periderm), peel (cortex), parenchyma and central vascular xylem bundle (IITA, 1990). The parenchyma, which is the edible portion of the fresh root, comprises approximately 85% of total weight, consisting of xylem vessels radially distributed in a matrix of starch containing cells (Wheatley and Chuzel, 1993). The peel layer, which is comprised of sclerenchyma, cortical parenchyma and phloem, constitutes 11-20% of root weight. The periderm (3% of total weight) is a thin layer of cells and, as growth progresses, the outermost portions usually slough off. Root size and shape depend on cultivar and environmental conditions. Variability in size between cultivars is greater than that found in other root crops (Wheatley and Chuzel, 1993).

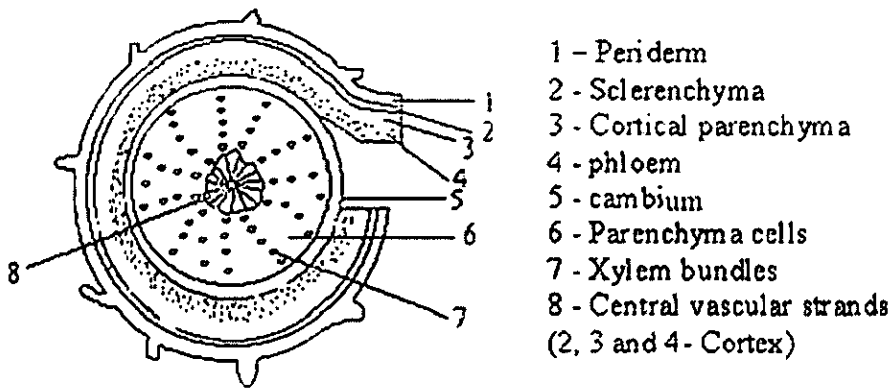

Figure 2.1 Cross-section of a cassava storage root (IITA, 1990)

2.2 PRODUCTION AND IMPORTANCE OF CASSAVA

2.2.1 Ecology

Cassava is a tropical crop, distributed between latitudes 30° N and 30° S (Costa and Silva, 1992; Alves 2002). The ideal growth temperature range is 24 to 30 °C (IITA, 1990) but it can tolerate temperatures ranging from 16 to 38 °C. Cassava can grow in the semi-arid tropics with an annual rainfall less than 600 mm, but the ideal rainfall is 1000 to 1500 mm per year (Alves, 2002). Cassava can grow in low-nutrient soils where cereals and other crops do not grow. It grows well in sandy to light soils where the storage roots can develop easily. Cassava can tolerate soils with low pH. Soils with a superficial hard layer or with many stones are not suitable for cassava growth.

2.2.2 Cassava production

The total world cassava production in the year 2000 was 172 million tonnes according to FAO (2002). Africa accounted for 54%, Asia for 28% and Latin America and the Caribbean for 19% (IITA, 2002). In 1999, Nigeria produced 33 million tonnes, making it the world's largest producer. In Africa, Mozambique was 5th in cassava production in 1998 with 5.6 million tonnes, after Nigeria, Democratic Republic of Congo, Ghana and Tanzania (FAO, 1998). The average world cassava yield in 2002 was 10.6 tonnes per ha. Maximum yield was reported to be 27.3 tonnes per hectare in Barbados (IITA, 2002).

2.2.3 Importance of cassava

Cassava has been described as a subsistence crop for rural householders, but it is increasingly becoming a cash crop in Africa (Nweke *et al.*, 2002), where the majority of cassava produced (88%) is used for human food (Westby, 2002). According to Nweke *et al.*, (2002), cassava

plays five important roles in African development: famine-reserve crop, rural staple food, cash crop for both rural and urban households and, to a minor extent, raw material for feed and chemical industries. In South America, it is used mostly for animal feed (about one-third) followed by human consumption then starch production. In Asia, the cassava production is dominated by exportation to the European Union for use in animal feed (Almeida, 1995; Westby, 2002). In Mozambique, cassava contributes 42 % of the total energy intake (Dahniya, 1994) and is the second important staple food after maize (INIA and SARRNET, 2003).

2.3 CHEMICAL AND NUTRITIONAL COMPOSITION OF CASSAVA

2.3.1 Chemical and nutritional composition of cassava roots

Cassava roots and cassava leaves are used for human consumption and animal feed (Buitrago 1990, Dahniya, 1994). The general chemical composition of cassava roots and leaves is shown in Table 2.1.

Table 2.1 Chemical composition of cassava roots and leaves (Buitrago, 1990)

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

Cassava roots are rich in digestible carbohydrates, mainly in starch. Cassava root starch consists of both amylose (20%) and amylopectin (70%) (Buitrago, 1990). There is a large variation in sucrose content between cassava genotypes. In sweet varieties, sucrose constitutes about 17% of total carbohydrates (Hendershott, unpublished paper). Generally, cassava roots have less than 1% free sugars (Bradbury & Holloway, 1988).

Cassava roots are low in protein and fat. A comparison of the protein composition of cassava roots with other foods is shown in Table 2.2. Cassava root has less than the recommended minimum limit in almost all essential amino acids, except tryptophan. Cassava roots should be eaten along with other crops rich in essential amino acids to supplement the deficit, such as vegetables, cereals, fish and meat. Cassava leaves are much richer in protein than the roots, although the leaf contains a lower proportion of methionine than the root protein. The levels of all other essential amino acids in leaf protein exceed the FAO's recommended reference (Okigbo, 1980; FAO, 1990).

Cassava has a high content of dietary fibre, magnesium, sodium, riboflavin, thiamine, nicotinic acid and citrate (Bradbury and Holloway, 1988). The major constraint on cassava roots as human food is the presence of toxic cyanogenic glucoside compounds in the tissues.

Table 2.2 Comparison of FAO (1990) protein amino acid patterns with amino acid composition of protein of cassava and other foods

Amino acid	(g/ 100 g crude protein)					
	Cassava roots*	Potato*	Sweet potato *	cowpea †	Egg*	FAO Reference patterns*
Lysine	4.14	6.00	3.40	6.83	7.00	5.50
Threonine	2.64	3.90	3.80	3.60	4.70	4.00
Tyrosine+ phenylalanine	4.10	7.80	6.20	7.78	9.30	6.00
Valine	3.34	5.10	4.50	4.53	6.60	5.00
Tryptophan	1.15	1.40	1.40	1.09	1.70	1.00
Isoleucine	2.80	3.90	3.70	3.82	5.40	4.00
Methionine+cysteine	2.77	3.00	2.80	2.26	5.70	3.50
Leucine	4.00	5.90	5.40	7.74	8.60	7.00
Total Amino acids	24.94	36.30	31.20	37.65	49.00	36.00

* Modification from FAO, 1990
† (FAO, 1990), except Leucine (Bressani, 1985)

2.4 CYANOGENIC GLUCOSIDES

2.4.1 Cyanogenic glucosides in the plant kingdom

Cyanogenic glucosides are phytotoxins that occur in about 2000 plant species, of which a number are used as crops (Conn, 1980). Cassava and sorghum are especially important staple foods containing cyanogenic glucosides. It is believed that cyanogens confer protection against attack by some herbivores (Bellotti and Riis, 1994).

Cassava contains, in all the tissues, with the exception of the seeds, 4 to 5 cyanogenic glucosides. The main ones are linamarin and lotaustralin in a ratio 97:7 (Teles, 1995). The concentrations of cyanogens differ in different varieties, between tissues in the same plant and even between compartments of the same tissue (Bruijn, 1971; Sundaresan *et al.*, 1987). Cassava tissues also contain the enzyme linamarase, which can hydrolyse cyanogens but the enzyme is

not in the same compartments as the cyanogens (Bruijn, 1971: Nweke, 1994: Teles, 1995). Cyanogenic glucosides are located inside vacuoles and the enzyme linamarase in the apoplastic (Conn, 1994).

2.4.2 Distribution of cyanogenic glucosides in cassava plants

Cassava leaves have the highest concentration of cyanogens. In the leaves, the concentration decreases with age (Bruijn, 1971; Teles, 1995). In new leaves the cyanogen levels are higher in the lamina than in the petiole, but in old leaves the petiole has a higher cyanogen level than the lamina (Bruijn, 1971). In cassava roots, in the longitudinal direction, cyanogen concentration increases from insertion point on the plant to the root terminal and in the transverse direction, cyanogen levels decrease from the external area to centre of the root (Bruijn, 1971).

Cassava varieties are often described as being bitter or sweet by reference to the taste of fresh roots and this partly correlates with cyanogen concentrations. Bitter varieties are associated with high concentrations of cyanogenic glucosides (Sundaresan *et al.*, 1987), while sweet varieties have a high concentration of free sugars but it does not always follow that they have low concentrations of cyanogens (Borges & Fukuda, 1989). However, bitter taste and high level of cyanogens can also be related to environmental stress conditions, such as drought, low soil fertility and pest attack (Bruijn, 1971).

2.4.3 Biosynthesis of cyanogenic glucosides

Cyanogenic glucosides are a group of amino acid derived secondary metabolites in the plant kingdom. In the cassava plant the two main cyanogens, linamarin and lotaustralin, are synthesized from L-valine and L-isoleucine respectively (Figure 2.2). The mechanism involved in the biosynthetic pathway for the formation of glucosides is a four electron oxidative decarboxylation of the amino acid where, in the first step, the formation of an oxime is

catalysed by a multi functional P-450 cytochrome (Andersen *et al.*, 2000). The oxime is dehydrated to form a nitrile, followed by the oxygenation of that nitrile to form an α-hydroxynitrile. The final step is thought to involve the glucosylation of the α- hydroxynitrile by a biological glucosylating reagent (Conn, 1994).

2.4.4 Hydrolysis of cyanogenic glucosides

Disruption of cassava tissues initiates the hydrolysis of cyanogens, which come into contact with linamarase, a β-glucosidase, to produce acetone cyanohydrin from linamarin and 2-butanone cyanohydrin from lotaustralin (Conn, 1994).

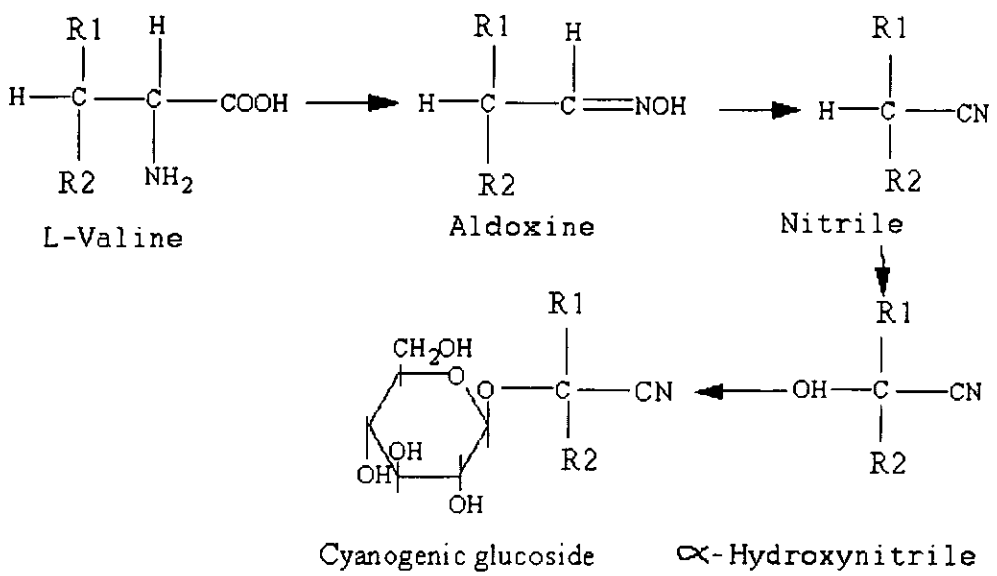

Figure 2.2 The generalized biosynthetic pathway for cyanogenic glucosides as proposed by Hahlbrock *et al.* (1968). Linamarin ($R_1 = R_2 = CH_3$) and lotaustralin ($R_1 = C_2H_5$, $R_2 = CH_3$)

These cyanohydrins are unstable and decompose spontaneously to the corresponding ketones and hydrogen cyanide at pH values above 5 and temperatures above 30 °C. Cyanohydrin degradation (Figure 2.3) can also be catalysed by α-hydroxynitrile lyase, located in apoplastic (White *et al.*, 1994).

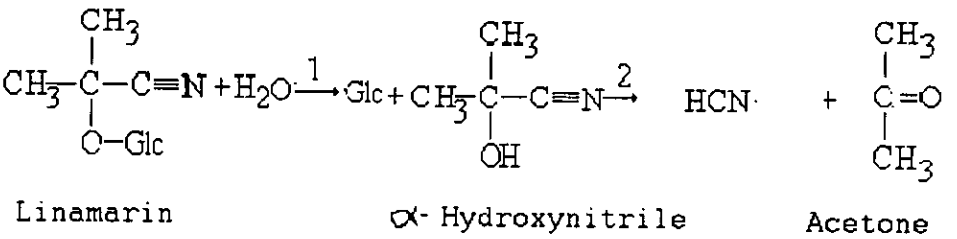

Figure 2.3 Hydrolysis of linamarin, Glc = glucose, 1 - ß – glucosidase (pH = 5.5)
2 - α-hydroxynitrile lyase (pH ≥ 5) (Conn, 1994)

2.4.5 Toxicology of cyanogenic of cassava

The presence of cyanogenic glucosides in cassava tissues is related to various health disorders that occur in populations where cassava is the staple food. These disorders include tropical ataxic neuropathy, epidemic spastic paraparesis, also known as *konzo* (Rosling, 1986; Rosling, 1988), endemic goitre and cretinism (Delange *et al.*, 1994). These problems have been reported in the Democratic Republic of Congo, Nigeria and Mozambique.

Such health disorders occur when there are prolonged cyanide exposures associated with food shortage, social instability, under-nourishment and deficiency in some essential nutrients such as iodine and essential sulphur amino acids (Delange *et al.*, 1994; Teles, 1995).

Cases of fatal and acute intoxication have been reported. In 1887, the crew of Stanley's remarkable expedition through the Democratic Republic of Congo, suffered from fatal acute poisonings when they consumed bitter cassava roots without the extensive soaking as applied

by local inhabitants (Manning, 1985). In Nampula-Mozambique, Ceara-Brasil and Nigeria acute toxic effects after consumption of cassava meals have been reported (Ministry of Health, 1984; Akintonwa *et al.*, 1994; Teles, 1995). The acute oral lethal dose of hydrogen cyanide (HCN) is 3 mg/kg body weight (Borges and Fukuda, 1989). The HCN blocks the reduction of oxygen in the respiratory pathway (Lehninger, 1982). The FAO/WHO (1991) recommended limit value for the safety of consumption of cassava products is 10 mg HCN/kg.

Cassava cyanogens can be reduced to low levels by several cassava processing methods and when cassava is eaten with other foods to balance the nutritional value, there is little danger of intoxication (Teles, 1995; Westby, 2002).

2.4.6 Mechanisms of cyanogen detoxification by the human body

Residual cassava cyanogens in processed cassava products, when ingested, are hydrolysed in the human digestive system. It is assumed that the cyanogens are hydrolysed by intestinal microbes (Teles, 1995). The HCN in the human body is metabolised to thiocyanate (McMahnon and Birnbaum, 1990). The conversion of HCN to thiocyanate is catalysed by rhodanese and 3-mercaptopyruvate sulphur transferase (Westley, 1981; Vazques *et al.*, 1987). These enzymes require sulphur, which is supplied from sulphur amino acids. The thiocyanate (SCN) is eliminated from the human body through urine and saliva (Figure 2.4).

Excessive levels of SCN may lead to reduced iodine uptake, which, in an iodine-deficient region, may contribute to endemic goitre and cretinism (Bradbury and Holloway, 1988; Delange *et al.*, 1994). Figure 2.4 shows a scheme of the metabolism of cyanide to thiocyanate in the human body.

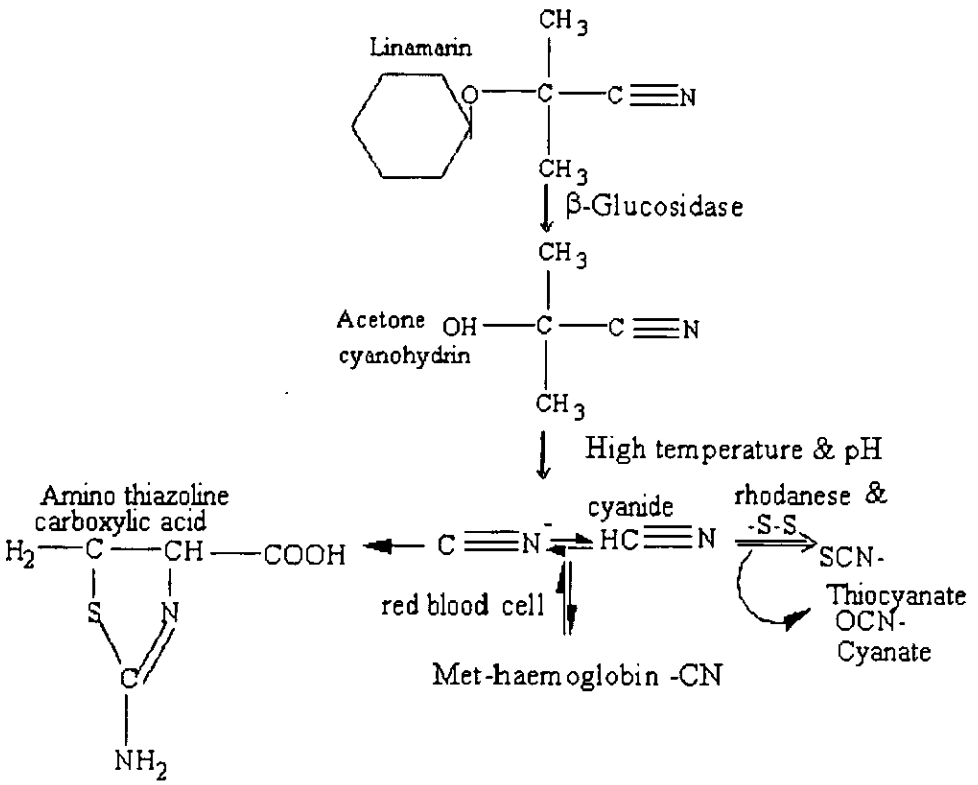

Figure 2.4 Metabolism of cyanide to thiocyanate in the human body (Rosling, 1994)

2.4.7 Methods for the analysis of cyanogens

There are many different methods that have been developed for the determination of cyanogens in cassava (Bradbury *et al.*, 1994). All methods of analysis of cyanogens in cassava involve mainly three steps: extraction of cyanogens from cassava, hydrolysis to cyanide and analysis of cyanide (Borges *et al.*, 1993; Bradbury *et al.*, 1994).

Extraction of cyanogens from the plant material is normally carried out using dilute acid, 0.1 M phosphoric acid (Cooke, 1978; AOAC, 1990; Essers *et al.*, 1993). The hydrolysis of cyanogens to cyanide can be done either by autolysis (Bradbury *et al.*, 1994), by enzymatic hydrolysis (Cook, 1978; Essers *et al.*, 1993), or by acid hydrolysis (Bradbury *et al.*, 1991).

Autolysis, which consists of hydrolysis of linamarin by the endogenous linamarase present in the tissues, is slow and can extend to 24 hours (Bradbury *et al.*, 1994). In enzymatic hydrolysis, linamarase is added to the acid extracted suspension and the pH adjusted to 6. The breakdown of cyanogenic gluosides occurs in 15 min at 30 oC (Cooke, 1978; Nambisan, 1999; Haque and Bradbury, 1999). The enzyme can be produced from cassava root cortex or cassava latex (Nambisan, 1999). The acid hydrolysis method involves the hydrolysis of cyanogens in 2M H_2SO_4 at 100 °C for 50 min. The enzymatic and acid hydrolysis methods convert linamarin to cyanohydrins. The cyanohydrins are decomposed to cyanide by increasing the pH to alkaline levels (Bradbury *et al.*, 1994).

For the determination of cyanide, various methods have been developed, and the following methods are used:

2.4.7.1 Titration method

The titration method consists of autolysis of cassava samples followed by steam distillation to isolate the cyanide and, finally, titration with $AgNO_3$ (AOAC, 1990). This method has the disadvantage of low conversion of linamarin to cyanohydrin during autolysis and loss of cyanide during distillation (Cooke and De la Cruz, 1982, Borges *et al.*, 1993).

2.4.7.2 Alkaline picrate method

The alkaline picrate method is a semiquantitative method in which cyanide reacts with alkaline picrate paper and the change of colour is matched with a colour chart (Williams and Edwards, 1980). For more accurate cyanide determination, the picrate paper after reaction with cyanide is immersed in water and the absorbance of the solution is measured at 510 nm (Bradbury *et al.*, 1999). This method has been recently modified to allow determination of the three forms of

cyanogens present in cassava (HCN, acetone cyanohydrin and linamarin) (Bradbury *et al.*, 1999).

2.4.7.3 Colorimetric method

The colorimetric method is the most important and most accurate method. The method is based on the König reaction in which CN^- is oxidized to a cyanogen halide by chloramine T (Bradbury *et al.* 1994). The cyanogen halide is reacted with pyridine or a related compound to produce a dialdehyde, which is then coupled with primary amines or compounds with active methylene groups such as pyrazolone or barbituric acid to give a colored complex (Lambert *et al.*, 1975). For development of colour, the following combination of reagents have been developed: pyridine/pyrazolone (Cooke, 1978), pyridine/barbituric acid (Mendoza *et al.*, 1984; Bradbury *et al.*, 1991), isonicotinic acid/barbituric acid (Nagashima, 1978; Meeussen *et al.*, 1989) and isonicotinic acid/dimethyl barbituric acid (Essers *et al.*, 1993). With isonicotinic acid/dimethyl barbituric acid the absorbance of the blue colored solution is measured at 605 nm (Essers *et al.*, 1993).

2.5 CASSAVA PROCESSING

2.5.1 Cassava post harvest deterioration

The second major constraint in the consumption of cassava roots, after its cyanogens content, is that fresh cassava roots deteriorate rapidly. Cassava roots have a shelf life of 24 to 48 h (Hillocks, 2002; Westby, 2002). Two types of cassava deterioration have been described: primary physiological deterioration that involves internal discoloration, and secondary microbial spoilage (Booth and Coursey, 1974). Physiological deterioration is a complex process, which is still not fully understood, involving wound response, such as increase in

enzyme activity (Westby, 2002). Traditional methods of root storage after harvesting, such as burial in soil and piling in shades, are used for small quantities of cassava roots for 3 to 7 days (Westby, 2002).

2.5.2 Processing of cassava roots

Cassava roots are used for human food and animal feed in a large number of different products. There are various processing methods used to produce different food products, depending on locally available processing resources, local customs and preferences (Hillocks, 2002). Cassava processing improves palatability, increases shelf life, facilitates transport and, most importantly, detoxifies cassava roots by removal of cyanogens (Nweke, 1994). The most commonly used methods of cassava root processing are boiling of the fresh roots, sun drying and fermentation.

2.5.2.1 Boiling

Boiling is used in the processing of cassava roots in almost all countries where cassava is used as food. It is used only for sweet cassava varieties with low levels of cyanogenic glucosides. The efficiency of removal of cyanogens during boiling is influenced by the ratio of roots to water. The increase in the volume of water increases the leaching out of cyanogens (Nambisan and Sundaresan, 1985). Boiling is less efficient in removal of cyanogens than sun drying.

2.5.2.2 Sun drying

Sun-dried products are the most common types of cassava processed products in Africa (Westby, 2002). The process involves peeling cassava roots, chipping (or grating) and spreading on dried grass or on the roof for sun drying. The efficiency of removal of cyanogens in this process depends mainly on the rate of moisture loss. Fast drying results in lower cyanogen removal, while slower rates of drying, which occur in large pieces of cassava or

whole roots, result in a higher reduction of cyanogenic glucosides (Nambisan & Sundaresan, 1985; Essers, 1996).

2.5.2.3 Fermentation

Fermentation is an important processing technique for cassava, especially in Africa. Three major types of the fermentation of cassava roots are recognized: the grated root fermentation, fermentation of roots under water and mould fermentation of roots in heaps (Westby, 2002).

The grated root fermentation method is important in the processing of many West African products, including roasted granules (*gari*), steamed granules (*Iattieke*) from Côte d'Ivoire and some of the fermented pastes (*agbelina* and *placali* from Ghana and Côte d'Ivoire respectively) (Westby and Choo, 1994, Westby, 2002). The grated root fermentation is also used in the south of Mozambique to produce *rali,* which is similar to *gari* (Francisco *et al.*, 1992). The grated cassava roots are allowed to ferment in sacks for 3-7 days, which encourages lactic acid fermentation. The pH after 3 days decreases from 6 to 4 and the fermentation is dominated by lactic acid bacteria (Westby and Twiddy, 1992). Grating is important for bringing linamarin into contact with linamarase allowing its hydrolysis to glucose and cyanohydrin and then to HCN (Westby and Choo, 1994). The hydrolysis continues during the fermentation process. Lactic fermented products are reported to have significant concentrations of cyanohydrin because pH decreases during fermentation and cyanohydrin is stable at low pH (Vasconcelos *et al.*, 1990). The processes of *gari* production reduce cyanogen contents by more than 95% (Westby, 2002).

Fermentation of cassava roots under water, followed by sun drying, is reported to be the best for cyanogen removal (Westby, 2002, Cardoso *et al.*, 2005). This type of fermentation is used more in areas where there is a sufficient supply of water such as near a river or lake, and is

common in countries such as Nigeria, Democratic Republic of Congo, Tanzania and Malawi (Westby, 2002).

Heap fermented cassava root products are produced in Tanzania (Ndunguru *et al.*, 1999), Uganda, and Mozambique (Essers *et al.*, 1995a). The heap fermentation was investigated in Uganda by Essers *et al.*, (1995a). The process involves peeling of cassava roots, sun drying for 1 to 3 days, heaping and covering, fermentation, scraping off the moulds, crushing into crumbs, sun drying, pounding and sieving into flour. During the fermentation of the roots, the temperature inside the heaps was 2 to 12 °C higher than outside the heaps where the temperature was 23 to 29 °C. Essers *et al.* (1995a) observed that the heap fermentation was dominated by mould mycelium growth of *Neurospora sitophila*, *Geotrichum candidum* and *Rhizopus oryzae*. Heap fermentation of cassava roots followed by sun drying is capable of reducing the cyanogen levels by 95% (Essers *et al.*, 1995a).

2.5.3 Biochemistry of fermentation of cassava roots and the role of bacteria and fungi

Fermentation is the metabolic process in which carbohydrates and related compounds are broken down by oxidation, with the release of energy, in the absence of any external electron acceptors (Jay, 1992). Fermentation is a general term denoting the anaerobic degradation of glucose or other organic nutrients into various products (characteristic for different organisms) for the purpose of obtaining energy (Lehninger, 1982). The most common products of fermentation are ethanol from alcoholic fermentation and latic acid from latic acid fermentation and the overall reactions are as follows (Lehninger, 1982):

$$\text{Glucose} + 2P_i + 2\text{ADP} \longrightarrow 2\ \text{ethanol} + 2CO_2 + 2\text{ATP}$$

$$\text{Glucose} + 2\ P_i + 2\ \text{ADP} \longrightarrow 2\ \text{Lactic acid} + 2\ \text{ATP}$$

Lactic acid fermentation is carried out by lactic acid bacteria and alcoholic fermentation by yeast. In food processing, fermentation is an important process where the product becomes more stable, and acquires aroma and flavour appreciated by the consumers. The fermentation process, in some instances, increases the nutritional value and increases digestibility and reduces the toxicity of some foods (Jay, 1992).

Cassava fermentation involves various microbes, lactic acid bacteria, yeasts and moulds, depending on different fermentation techniques and preferences (Westby and Choo, 1994; Essers *et al.*, 1995a). The microbes are involved in the degradation of cassava carbohydrates and produce different products with different flavours and tastes improving the preservation of cassava products (Jones *et al.*, 1993). Most importantly, the microbes are involved in the reduction of cyanogenic glucosides in cassava roots (Westby, 2002).

Westby and Twiddy (1992) observed a decrease in fructose, glucose and sucrose and an increase in latic acid during fermentation of grated cassava roots. In grated roots, lactic acid bacteria play no role in cyanogen reduction (Westby and Choo, 1994). Lactic fermentation of grated cassava roots improves the taste and flavour of cassava in the final product, *gari*.

In soaked roots, microbial growth is essential because it enables softening of the roots which has the combined effect of allowing linamarin to come into contact with linamarase and also enables leaching of the cyanogens (Westby and Choo, 1994). The fermentation of soaked roots to produce *fufu* is dominated by lactic acid bacteria with a decrease of pH during the fermentation (Westby and Twiddy, 1992).

Fermentation of cassava roots using moulds is less documented. Essers *et al.* (1995a) observed that the growth of moulds softens the roots, which is presumably enhanced by cell wall degrading enzymes produced by the moulds. The degrading of cassava roots enables contact between linamarin and linamarase. The pH during the heap fermentation was between

5.5 and 6.3, which is optimum for linamarase activity (Nok and Ikediobi, 1990). The high pH at the end of heap fermentation enhances the breakdown of cyanohydrin into volatile HCN (Essers *et al.*, 1995a).

2.5.4 Mycotoxins in cassava products

Mycotoxin producing fungi may grow during some processing stages of cassava roots, such as heap fermentation, drying and storage. When the growth of potentially mycotoxigenic fungi occurs, there is the possibility of formation of mycotoxins (Westby, 2002). A number of potentially mycotoxigenic storage fungi have been isolated from cassava and mycotoxin contamination of cassava has been documented but the potential risk with this contamination has not been fully assessed (Westby, 2002). Essers *et al.* (1995a) reported that no mutagenic or cytotoxic compounds were detected and aflatoxins were absent in heap fermented cassava samples from Uganda.

2.5.5 Improvement of protein content of cassava products using microbial techniques

Several micro-organisms have been investigated to increase the protein content of cassava and cassava residues using solid-state fermentation (Raimbault *et al.*, 1985; Daubresse *et al.*, 1987; Noomhorn *et al.*, 1992; Zvauya and Muzondo, 1993).

In studies on the protein enrichment of cassava by solid state fermentation, dried cassava roots were moistened, mixed with a nitrogen source, such as urea or ammonium sulphate, and then inoculated with moulds, such as *Rhizopus oryzae* or *Aspergillus niger*. During the fermentation, the moulds produced protein, increasing the protein content in the fermented product (Dubresse *et al.*, 1987; Noomhorm *et al.*, 1992). Protein enrichment during fermentation occurs also due to losses in total dry matter, specifically starch (Dubresse *et al*, 1987; Balagopalan and Padmaja, 1988; Zvauya and Muzondo, 1993). Dubresse *et al.* (1987)

showed that fermentation of cassava mixed with a nutritive solution consisting of urea, potassium dihydrogen phosphate and magnesium sulphate with *Rhizopus oryzae* increased protein concentration of cassava flour by 68%.

Cassava root products can also be enriched using a combination of the fermentation process and addition of known protein rich food crops such as soybeans or cowpea (Oyewole and Aibor, 1992; Sanni and Sobamiwa, 1994; Mjimba, 1998). Protein enrichment in solid-state fermentation of cassava roots has an additional benefit of detoxifying of cyanogens (Birk *et al.*, 1996).

In this study, an attempt was made to use a combination of fermentation using selected moulds and cofermentation of cowpea flour with fresh cassava roots to increase the protein content in cassava. Cowpea is a legume of major dietary and economic importance in Mozambique. It contains 24% protein (w/w) and is relatively cheaper than other legumes. Cowpea is rich in lysine and consequently it can be used to enrich cereals. Like cassava, cowpea is deficient in sulphurous amino acids, but compared to other legumes, methionine and tryptophan levels are high (Lambot, 2002). Except for total sulphurous amino acids, and to a lesser extent isoleucine, levels of essential amino acids are at least as high as those in soybean. The digestibility of cowpea protein is 80% (Carnovala *et al.*, 1990). Cowpea flour can be processed fairly easily without defatting before milling due to its low fat content (1.1-3.0% w/w), (McWatters *et al.*, 1995). Cowpea contains a higher level of flatulent sugars but its raffinose content is lower than that in soybean. Soaking the grain before dehulling and milling decreases levels of the flatulent sugars (Lambot, 2002). Therefore, the presence of flatulent sugars in cowpea should not limit its use. Levels of trypsin inhibitors in cowpea are about half the values observed in soybean and are inactivated by heating. The phytate content of cowpea is half that of soybean (Lambot, 2002).

Theoretical expected protein composition benefits of cassava roots supplementation with cowpea flour at proportions cassava roots: cowpea flour, 96 g: 4 g, 92 g: 8 g and 88 g: 12g are shown in Tables 2.3, 2.4 and 2.5.

Table 2.3 Expected amino acid balance when cassava:cowpea are mixed in proportion 96:4 g/g compared with cassava and cowpea alone

Amino acid (g/100g Of protein)	Cassava* alone	Cowpea** Alone	Mixture 96:4 (g/g)	FAO***	Protein chemical Score‡		
					Cassava	Cowpea	Mixture
Lysine	4.14	6.83	5.49	5.5	75.3	124.2	99.8
Threonine	2.64	3.60	3.12	4	66.0	90.0	78.0
Tyr + Phe	4.10	7.78	5.94	6	68.3	129.6	98.9
Valine	3.34	4.53	3.94	5	66.9	90.6	78.7
Tryptophan	1.15	1.09	1.12	1	115.2	108.8	112.0
Isoleucine	2.80	3.82	3.31	4	70.0	95.6	82.8
Met +Cysteine	2.77	2.26	2.51	3.5	79.1	**64.5**	**71.8**
Leucine	4.00	7.74	5.87	7.0	**57.1**	110.6	83.9
Total AA	**24.94**	**37.65**	**31.30**	**36.00**			
Total protein (g/100 g of sample)	**1.00**	**24.00**	**3.84**				

AA: amino acid

Source: * (FAO, 1990)
** (FAO, 1990), except Leucine (Bressani, 1985)
*** Recommeded level (FAO, 1990)
‡ (Cheftel, *et al.*, 1985)

Table 2.4 Expected amino acid balance when cassava:cowpea are mixed in proportion 92:8 g/g compared with cassava and cowpea alone

Amino acid (g/100g of protein)	Cassava* alone	Cowpea** alone	Mixture 92:8 (g/g)	FAO***	Protein chemical Score‡		
					Cassava	Cowpea	Mixture
Lysine	4.14	6.83	5.96	5.5	75.3	124.2	108.4
Threonine	2.64	3.60	3.29	4	66.0	90.0	82.2
Tyr + Phe	4.10	7.78	6.58	6	68.3	129.6	109.7
Valine	3.34	4.53	4.14	5	66.9	90.6	82.9
Tryptophan	1.15	1.09	1.11	1	115.2	108.8	110.9
Isoleucine	2.80	3.82	3.49	4	70.0	95.6	87.3
Met +Cysteine	2.77	2.26	2.42	3.5	79.1	**64.5**	**69.2**
Leucine	4.00	7.74	6.53	7.0	**57.1**	110.6	93.3
Total AA	**24.94**	**37.65**	**33.53**	**36.00**			
Total protein (g/100 g of sample)	**1.00**	**24.00**	**5.68**				

AA: amino acid

Source: * (FAO, 1990)
** (FAO, 1990), except Leucine (Bressani, 1985)
*** Recommeded level (FAO, 1990)
‡ (Cheftel, *et al.*, 1985)

Table 2.5 Expected amino acid balance when cassava:cowpea are mixed in proportion 88:12 g/g compared with cassava and cowpea alone

Amino acid in g/100g of protein	Cassava* alone	Cowpea** alone	Mixture 88:12 (g/g)	FAO***	Protein chemical Score‡		
					Cassava	Cowpea	Mixture
Lysine	4.14	6.83	6.20	5.5	75.3	124.2	112.8
Threonine	2.64	3.60	3.38	4	66.0	90.0	84.4
Tyr + Phe	4.10	7.78	6.91	6	68.3	129.6	115.2
Valine	3.34	4.53	4.25	5	66.9	90.6	85.0
Tryptophan	1.15	1.09	1.10	1	115.2	108.8	110.3
Isoleucine	2.80	3.82	3.58	4	70.0	95.6	89.6
Met +Cysteine	2.77	2.26	2.38	3.5	79.1	**64.5**	**67.9**
Leucine	4.00	7.74	6.87	7.0	**57.1**	110.6	98.1
Total AA	**24.94**	**37.65**	**34.67**	**36.00**			
Total protein (g/100 g of sample)	**1.00**	**24.00**	**7.52**				

AA: amino acid

Source: * (FAO, 1990)
** (FAO, 1990), except Leucine (Bressani, 1985)
*** Recommeded level (FAO, 1990)
‡ (Cheftel, *et al.*, 1985)

3.0 MATERIALS AND METHODS

3.1 CYANOGENIC POTENTIAL OF CASSAVA FLOUR SAMPLES COLLECTED FROM SELECTED DISTRICTS OF NAMPULA PROVINCE, MOZAMBIQUE

3.1.1 Study area

Four villages in four different districts that were not close to each other were chosen as study sites (Fig 3.1). The first study village was Acordos de Lusaka, Miaja, in Memba District, Nampula, Mozambique. The second study site selected was in Niyaro village, Nebala locality in Monapo District, Nampula, Mozambique. Niyaro village was a new settlement by a main road (N8) and most couples were young. Sun-dried cassava chips were being sold by the roadside. The third study site was in Quixaxe village, Quixaxe locality in Mogincual District, Nampula, Mozambique. The site was near Quixaxe clinic and Quixaxe high school. The fourth site was in Naconha village, Nametil locality in Mogovolas District, Nampula, Mozambique. Memba and Mogincual districts had been previously studied with respect to cyanogen levels of cassava flour from rural homes (Cardoso *et al.*, 1998; Ernesto *et al.*, 2000)

3.1.2 Sample collection

On arrival at each study site the research group was divided into two teams. Where possible, thirty flour samples were collected from different homes in each location in randomly selected directions. An attempt was made to collect a sample of flour that was being used for making a meal that day. The quantity of a full plastic cup of flour samples (250 ml) was collected in a plastic bag. The samples were collected during a period of 3 days and taken to a laboratory in Nampula City where cyanogenic potentials were determined as described in section 3.1.3.

Additional information was obtained on the cassava varieties grown and the processing methods.

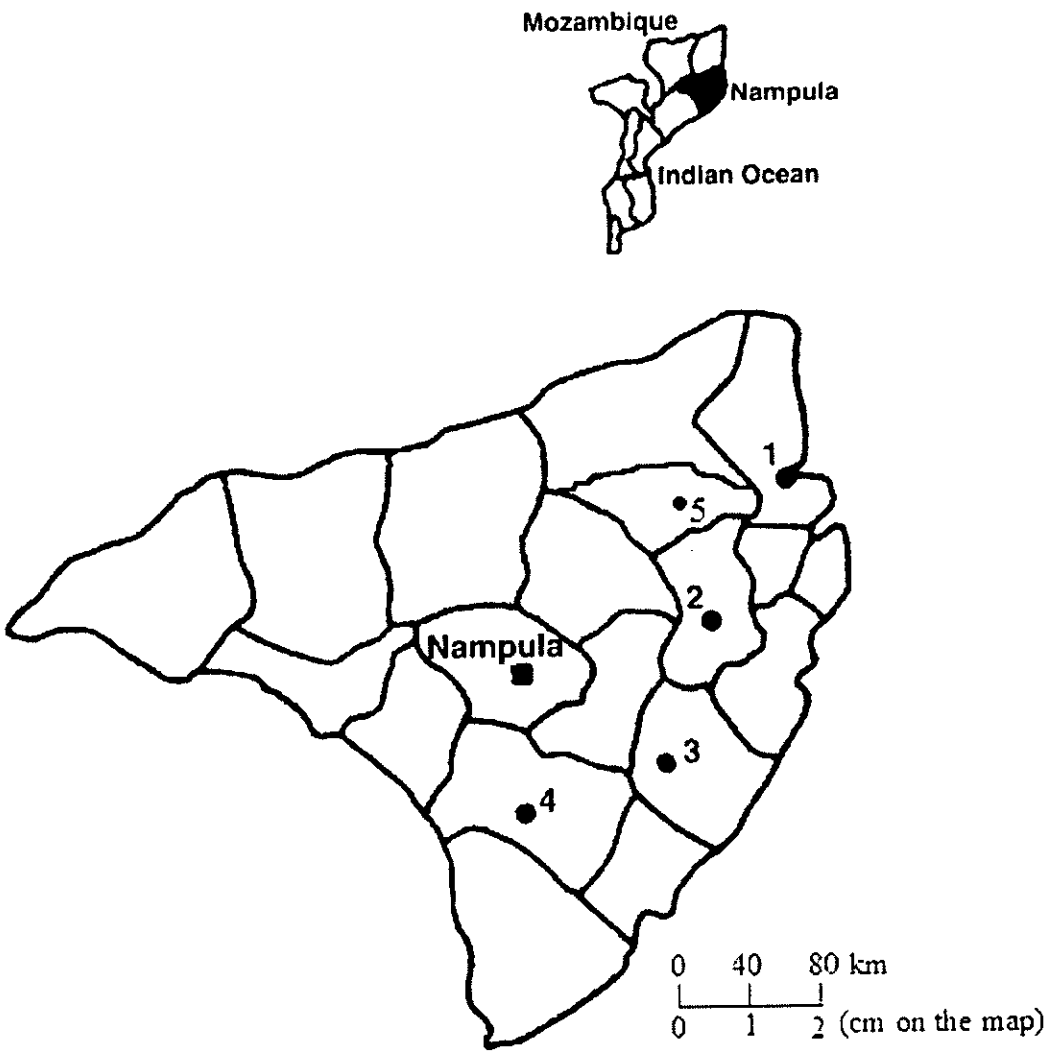

Figure 3.1. Map of Nampula Province, shown in relation of the rest of Mozambique: 1- Acordo de Lusaka, Memba District, 2 - Niyaro Village, Monapo District, 3- Quixaxe village, Mogincual District, 4- Naconha village, Mogovolas District and 5- Nacaroa village, Nacaroa district

3.1.3 Analysis of cyanogenic potential of cassava flour samples

The method used for the analysis of cyanogenic potential of cassava flour samples is based on the method described by Essers *et al.* (1993). The method allows the quantification of HCN by means of a reaction using isonicotinate/1,3-dimethyl barbiturate as a colour reagent.

3.1.3.1 Preparation of assay reagents

Sodium hydroxide (0.2 M), was prepared by dissolving NaOH (8 g) in distilled water (1 L).

Orthophosphoric acid (0.1 M) was prepared by adding phosphoric acid (85%, 6.78 ml) to distilled water (1 L) in a volumetric flask.

Phosphate buffer, pH 6, was prepared from phosphoric acid (0.1 M) and tri-sodium phosphate (0.1 M).

Chloramine T was prepared freshly each day by dissolving chloramine T (BDH UN 3263) (0.5 g) in distilled water (25 ml).

Colour reagent was prepared by adding NaOH (3.7 g), 1,3-dimethylbarbituric acid (Fluka RB 13883) (7.0g) and isonicotinic acid (Fluka RB 10846) (5.7 g) to distilled water (200 ml) and the solution was stirred intensively. The pH was adjusted to between 7 and 8 with NaOH (1 M) and the solution was stored at room temperature for up to 12 days.

The enzyme linamarase, used in the total cyanogens assay, was prepared from latex extracted from cassava leaves by the method described by Nambisan (1999) and Haque & Bradbury (1999). Latex from 20 to 30 cassava leaves was collected from the petioles after they were broken off from the main branches of cassava plants and added to distilled water (10 ml) in 25 ml centrifuge tubes. The mixture was shaken with a vortex mixer (Stuart) for 10 min and centrifuged at 2000 rev min^{-1} for 15 min. The supernatant enzyme solution was placed into plastic vials and frozen at –20°C.

3.1.3.2 Assay of enzyme activity

The assay of enzyme activity was based on the method described by Bradbury *et al.* (1999). Squares of Whatman 3MM paper (21 mm diameter) loaded with a known amount of linamarin, 40 µg HCN equivalent (Australia National University) were added to small flat bottomed plastic vials (25 mm diameter, 50 mm high) followed by 0.1 M phosphate buffer at pH 6 (0.5 ml), aliquots of prepared enzyme solution (100 µl) and picrate paper (prepared according Bradbury *et al.*, 1999). The plastic vials were immediately closed with screw caps and incubated at 30 °C for 5 hours. The picrate papers were removed from the vials, immersed in distilled water (5 ml) and shaken by a vortex mixer (Stuart) for about 10 min. The absorbance of the solutions was measured at 510 nm against a blank containing of phosphate buffer. The amount of HCN (µg) was obtained by multiplying the absorbances by 39.6 (Bradbury *et al.*, 1999). If the amount of HCN was between 20 to 40 µg, the enzyme activity was considered adequate for use in the determination of total cyanogens.

3.1.3.3 Extraction of cyanogens

Cassava flour samples (4 g) were mixed with a solution of 0.1 M phosphoric acid (25 ml) in screw cap plastic vials. The suspension was mixed by shaking three times by hand for about 15 min at intervals of 3 hours. The suspensions were then left to stand overnight. The clear supernatant was carefully pipetted into plastic vials. The extracts were stored at 4 °C until required for analysis.

3.1.3.4 Preparation of KCN Standard

A stock solution was prepared by dissolving KCN (BDH) (50 mg) in 0.2 M NaOH (50 ml). From this solution 0.2 ml was taken and diluted to 10 ml with 0.2 M of NaOH (solution B). From solution B, the following volumes were pippeted and placed into test tubes: 0.025, 0.050,

0.075 and 0.1 ml. The volume in each tube was made up to 0.1 ml with 0.2 M NaOH, to obtain 5, 10, 15 and 20 µg of KCN/ml. Phosphate buffer (0.1 M) pH 6 (0.5 ml) and 0.2 M NaOH (0.6 ml) were added to each tube. Colour development was determined by measuring absorbance as described in section 2.1.5. The concentration of KCN was plotted against absorbance to make the calibration curve.

3.1.3.5 Determination of cyanogenic potential

Determination of cyanogenic potential was assayed in duplicate using the procedure described by NRI (1996). For total cyanogenic potential (glycosides, cyanohydrins and HCN), sample extracts (0.1 ml) were added to 0.1 M phosphate buffer pH 6 (0.4 ml) in a stoppered test-tube, linamarase enzyme solution (0.1 ml) was added and the solution was incubated for 15 min at 30 °C. 0.2 M NaOH (0.6 ml) was added and after 5 min phosphate buffer pH 6 (2.8 ml) and chloramine T solution (0.1 ml) were added. The solution was mixed and left for 5 min at room temperature. Colour reagent (0.6 ml) was added. The solution was left for about 10 min and the absorbance was determined at 605 nm. Blanks containing 0.1 M phosphoric acid in place of sample extracts were used.

Cyanogenic potential (CNP) was calculated as follows:

$$CNP(mgHCN/Kg) = \frac{A \times V \times 0.4151}{b \times W}$$

Were:

V: Volume of solution used for extraction (ml)
W: Sample weight (g)
A: Sample absorbance
b: slope, calculated from the KCN (µg/ml) against absorbance calibration curve

3.1.4 Moisture and dry matter content.

Cassava flour samples (2 g) were dried in an oven at 105 °C to constant weight, minimum time 3 hours (NRI, 1996). The percentage of moisture and dry matter content were calculated as follows:

% moisture = {(loss of weight)/initial sample weight}x100

% dry matter = 100 - % moisture.

3.1.5 Data treatment

The means of potential cyanogenic content between fermented and non fermented samples and between location were compared statistically using the two-tailed Student's *t*-test.

3.2 MICROBIAL AND BIOCHEMICAL CHARACTERISTICS OF CASSAVA HEAP FERMENTATION

3.2.1 Study area

The fieldwork was carried out in Nacaroa District, Nampula Province, Mozambique. Nacaroa is located about 160 km North East from Nampula city, in the North of Mozambique, about 2000 km from the capital city, Maputo (Figure 3.1). The district is 3593 km^2 with about 86 000 inhabitants.

3.2.2 Traditional heap fermentation

The fermentation process was observed in three households located 3.4 km, 4.3 km and 7.1 km from Nacaroa Town respectively.The fermentation processes were followed for a period of 5

days from the onset until the end of fermentation. pH values were measured daily with a portable pH meter (SCHOTT handy lab1). Temperature was measured inside and outside the heap twice a day, in the morning (between 7 and 8 o'clock a.m.) and in the afternoon (between 1 and 2 o'clock p.m.) throughout the fermentation. Samples were collected daily from the heaps and sun dried to moisture below 11% (w/w) at approximately 30 to 34° C for cyanogens and crude protein determination.

3.2.3 Enumeration of microbes

For samples from heap fermentations, microbial analysis was based on methods published by NRI (1996). Cassava samples collected at various intervals during fermentation (1 g) were placed into peptone water (Oxoid, CM0009) (9 ml), shaken, and serial dilutions were made. For enumeration of micro-organisms, each suspension of peptone water (0.1 ml) was used as inoculum for spread plating. Total aerobic mesophilic bacteria were determined using Total plate count agar (Oxoid, CM0325), lactic acid bacteria using Man-Rogosa-Sharpe (MRS) agar (Oxoid, CM0361) and yeasts and moulds using Wort agar (Oxoid, CM0247). Plates were incubated under aerobic conditions for 3 days at room temperature (30-32 °C), after which colony counts were carried out.

3.2.4 Isolation of microbes

Small pieces (1g) of fermented cassava collected from heaps at different stages of fermentation or dry fermented cassava were added to test tubes with peptone water (9 ml) (Oxoid, CM0009). The contents of the test tubes were mixed by shaking and each suspension (0.1 ml) was then inoculated and spread on Wort agar (Oxoid, CM0247), Malt Extract agar (Oxoid) and Man-Rogosa-Sharpe (MRS) agar (Oxoid, CM0361) plates. Cultures were incubated for 3 days at 30 °C. The most frequently occurring microbes were isolated and purified by repeated streaking

of single colonies on the same media. The isolated bacteria and yeast were preserved in 30 % glycerol at – 80 °C. Moulds were preserved on Malt Extract agar plates at 4 °C.

3.2.5 Identification of microorganisms

3.2.5.1 *Identification of bacteria*

Isolated bacteria stored at – 80 °C were transferred to a -20 °C freezer and kept there over night. The following day, after thawing at 4 °C, the bacteria were regenerated on APT agar (DIFCO 0654-17-0), by plating 0.1 ml of preserved suspension and incubating for 2 days at 30 °C. Cultures were observed under the microscope (1000x, Olympus, BH2) and their cell morphology described. The bacteria were inoculated into APT broth (DIFCO 0655-17-9) and incubated at 30 °C. The final pH was measured after 48 h. The catalase test was determined by emulsifying a loopful of growth on a plate with a drop of hydrogen peroxide (3 %) on a slide and observing effervescence (Harrigan & McCance 1966). Bacterial isolates were sent to the Science & Technology Centre at The University of Reading for molecular characterisation. RNA gene sequencing and PCR (Polymerase Chain Reaction) direct sequencing were carried out at the Science & Technology Centre.

3.2.5.2 *Identification of moulds*

Isolated moulds were regenerated on Potato dextrose agar plates (Oxoid, CM0139) and their growth characteristics and reproductive structures were observed under the microscope (Olympus, BH2 100x). A classification key was used for identification (Pitt & Hocking, 1997).

Isolated yeasts were also regenerated on potato dextrose agar (Oxoid, CM0139) but no attempt was made to identify the genera. The yeasts were distinguished from moulds by morphological and microscopic examination of colonies and cells.

3.2.6 Determination of cyanogenic potential

For fresh cassava roots, 3 roots from each household were sliced twice longitudinally and one piece of each root was cut into smaller pieces. A sample (15 g) was collected and added to 0.1 M phosphoric acid (25 ml) in a 50 ml centrifuge tube. For dried cassava chips, 3 chips were crushed using pestle and mortar. A flour sample (5 g) was collected and added to 0.1 M phosphoric acid (25 ml) in a 50 ml centrifuge tube. In the laboratory, the centrifuge tube contents were placed into a measuring cylinder and 0.1 M of phosphoric acid was added up to 100 ml. The mixture was transferred to a blender and homogenized 3 times for 1 min at intervals of 1 min and then the sample (25 ml) was centrifuged for 10 minutes at 2500 rev min^{-1}. The supernatant was carefully pipetted into plastic vials and stored at 4 °C until required for analysis. Cyanogenic potential in samples were determined on the supernatants as described in section 3.1.3.5.

3.2.7 Determination of Total Crude Protein

Crude protein was determined by the Kjeldahl method revised by NRI (1996) for assessing quality characteristics on non-grain starch staples.

3.2.7.1. *Preparation of reagents*

Kjeldahl catalyst solution was prepared by dissolving copper (II) sulphate pentahydrate (BDH) (2.5g) and selenium dioxide (BDH) (2.5 g) in distilled water (250 ml) in a one litre conical flask. Concentrated sulphuric acid (BDH) (250 ml) was added slowly to the mixture, cooling the flask in a bath of running tap water. The solution was stored in a closed glass bottle.

Sodium hydroxide solution, 50% (w/v), was prepared by dissolving NaOH (250 g) in distilled water (250 ml).

Boric acid, 2% (w/v), was prepared by dissolving boric acid (20 g) in distilled water (one litre) in a 1 L glass bottle.

Bromocresol green solution was prepared by dissolving bromocresol green (BDH) (250 mg) in distilled water (250 ml).

Methyl red solution, methyl red ((BDH) (50 mg) was dissolved in distilled water (250 ml).

Sodium citrate solution was prepared freshly each day by dissolving sodium citrate (BDH) (10 g) in distilled water (1 L).

3.2.7.2 *Digestion of sample*

Cassava flour sample (2 g), potassium sulphate (2 g) and Kjeldahl catalyst (10 ml) were added to digestion tubes. The digestion tubes were placed in a support in a digestion infra-red heating block (Marconi). The mixture was heated until the solution became clear and light green (about 5 hours). The tubes were cooled and sodium citrate solution (25 ml) was added to each tube. The tubes were stored at room temperature over night and the samples were distilled the following day.

3.2.7.3 *Distillation of digested sample*

Each digestion tube with digested solution was connected to the semi-automatic Kjeldahl distillation system (Marconi). Boric acid solution (2%) (25 ml), bromocresol green solution (4 drops) and methyl red solution (4 drops) were added to each 250 ml receiver conical flask. Sodium hydroxide (50%) (25 ml) was added to the digested sample solution, and distilled until about 100 to 150 ml of distillate was collected in the receiver conical flask.

3.2.7.4 *Titration of distillate samples*

The distillate was titrated with hydrochloric acid (0.05 mol l^{-1}) until the indicator colour changed from green to grey to pink. Percentage crude protein was calculated as follows:

$$\% \text{ Crude protein } = \frac{0.07x6.25xA}{S} x100\% \text{ (NRI, 1996)}$$

A = HCl titration volume of sample (ml) - HCl titration volume of blank (ml).

S = Weight of sample (g)

3.2.8 Determination of pH

Cassava flour samples (5 g) were suspended in distilled water (20 ml) and the pH was determined with a portable pH meter (SCHOTT handy lab1). The pH meter was calibrated using standard buffers at pH 4 and pH 7.

3.3 LABORATORY SIMULATION OF CASSAVA HEAP FERMENTATION USING ISOLATED MOULDS

3.3.1 Preparation of cassava

Cassava roots acquired at a local market in Reading, UK (about 10 kg) were washed with water, left for 20 min to dry, cleaned with alcohol (70% v/v) using cotton wool and then peeled to remove the periderm and cortex . The sizes of cassava root slices were:

Cassava roots, 8 cm long

Cassava roots, 8 cm long, sliced longitudinally once

Cassava roots, 4 cm long

Cassava roots, 4 cm long, sliced longitudinally once

After slicing, all pieces were then cleaned again with alcohol (70% v/v) using cotton wool and left inside a laminar air flow cabinet for about 1 hour, until the surface of the roots appeared dry.

3.3.2 Preparation of mould spores

Spore suspensions of *Neurospora sitophila* (CNF7) and *Rhizopus stolonifer* (CNF6), isolated in Nampula province-Mozambique, were prepared as described by Teran & Owens (1996). The moulds were grown on Potato Dextrose Agar (Oxoid) (10 ml) in 250 ml Erlenmeyer flasks, incubated at 30 °C for 5 days. Spores were harvested by adding 20 ml sterile purified water (Purite RO50 reverse osmosis and ion exchange unit), scraping the mycelia with a sterile plastic loop to release the spores, agitating and filtering through a sterile nylon mesh (pore size 100 μm). The spores were washed three times in sterile purified water by centrifuging at 2000 rev min^{-1} for 10 min and decanting the supernatant. The spores were then suspended in 20 ml sterile purified water and stored in a refrigerator at 4 °C. The concentration of spores was determined by microscopic observation using a slide Counting Chamber (Assistent).

3.3.3 Fermentation of cassava slices

The cassava roots were placed in sterilized plastic containers and covered with sterilized tinfoil. The containers and tinfoil were sterilized by autoclaving at 121 °C for 15 min. Each size of cassava slices was inoculated separately with *Neurospora sitophila* and *Rhizopus stolonifer* (10^6 ml^{-1}) at a volume of 2 ml of the suspension per 500 g of roots. The samples were incubated at 30° C for 0, 24, 48 and 72 hours. In each container was placed two roots of each size, both for the longitudinally sliced and the unsliced roots. At 0, 24, 48 and 72 h, sampling and observation of cassava roots was carried out.

Uninoculated cassava roots of all the included sizes were also incubated as control. The experiment was carried out in triplicate for each treatment.

To check for the absence of bacteria on cassava roots, small pieces of cassava roots approximately 3x3x3 mm, were put in thioglycollate broth, incubated at 30 °C and observed after 72 hours.

3.3.4 Description of mould growth

The description of mould growth was carried out by visual observation of the cassava samples at each defined time of fermentation and score numbers were attributed as follows:

0- No growth (no mycelia), **1**- Slight growth (less than 1/4 of the roots covered with mycelia), **2**- Moderate growth (more than 1/4 and less than 1/2 of the roots covered with mycelia), **3**-Growth (more than 1/2 of the roots covered with mycelia), **4**- Good growth (all roots covered with mycelia).

3.3.5 Texture analysis of cassava roots during fermentation

Stable Micro Systems Texture Analyser (SMS - England) was used for analysis of the texture of cassava samples during fermentation. The following parameters were set on the equipment: test speed 0.5 mm/s, pre-test speed 10.0 mm/s, post-test speed 10.0 mm/s, trigger type 5.0 g and distance 5.0 mm. On each piece of cassava root, 5 points on the transversal and 3 points on the longitudinal side were measured and the final texture of the cassava piece was recorded as the average of the measured positions.

3.3.6 Determination of pH

The pH was measured during the fermentation process as described in section 3.2.8.

3. 4 PROTEIN ENRICHMENT OF CASSAVA BY CO-FERMENTATION WITH COWPEA

3.4.1 Preparation of cassava roots and preparation of cowpea

Freshly harvested *gangassol* variety cassava roots (20 kg) were washed with water, dried and cleaned with alcohol [70% (v/v)] using cotton wool. The periderm and cortex were removed, and the pulp was cut into pieces, approximately 10 cm in length. Cowpea grain, bought at a local market in Maputo, was milled and sieved through a 1 mm sieve. The composition of cowpea where: Dry matter 92%, protein 24.95% and ash content 3.10%. Lipids and carbohydrates were not determined.

3.4.2 Preparation of spores

Spore suspensions of *Rhizopus stolonifer* (CNF6), isolated in Nampula province-Mozambique, were prepared according to the method described by Teran & Owens (1996), as described in section 3.3.2.

3.4.3 Treatments of batches for fermentation

Cassava pieces were mixed with cowpea at different proportions (w/w) and either inoculated with *Rhizopus stolonifer* prior to fermentation or just fermented without fungal inoculation. Batches of fermented cassava were as follows:

1- Cassava, uninoculated

2- Cassava + cowpea (96:4), uninoculated

3- Cassava + cowpea (92:8), uninoculated

4- Cassava + cowpea (88:12), uninoculated

5- Cassava, inoculated

6- Cassava + cowpea (96:4) inoculated

7- Cassava + cowpea (92:8) inoculated

8- Cassava + cowpea (88:12) inoculated

The treatments were carried out in duplicate. Mixtures of cassava and cowpea (1kg) were placed into sterilized plastic containers. For inoculation, the pieces were sprayed with *Rhizopus stolonifer* spore suspension (10^6 spores ml^{-1}) (5 ml) in each container. The containers were placed in an incubator at 30 °C for up to 72 hours. Figure 3.2 shows the sequence for the experimental production of fermented cassava flour supplemented with cowpea.

3.4.4 Biochemical analyses

The pH was measured during the fermentation process as described in section 3.2.8.

Crude protein was determined in cassava flour samples as described in section 3.2.7 and dry matter was determined as described in section 3.1.4.

3.4.4.1 Determination of ash content

Total ash content was determined as described by NRI (1996). Cassava samples (3 g) were weighed into previously ignited, and cooled, and weighed silica dishes. The dishes and contents were gently heated over a low flame on bunsen burner until charred. The dishes were then transferred to a muffle furnace at 500 °C and left for about 5 hours. The dishes and contents were then cooled in a desiccator and reweighed. The total ash was calculated and expressed as a percentage:

% ash (w/w) = (Weight of ash/ dry weight of sample)x100

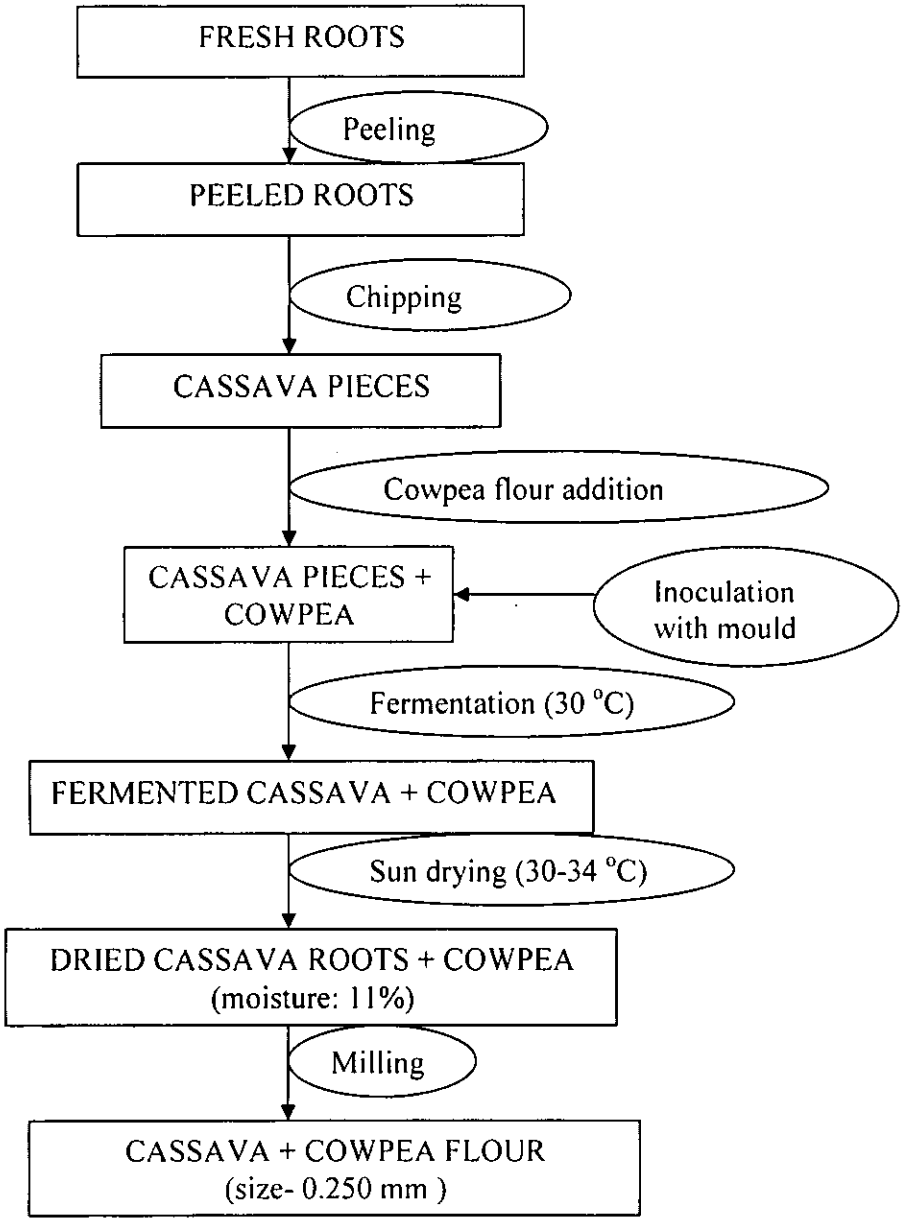

Figure 3.2: Sequence of experimental production of fermented cassava flour supplemented with cowpea

3.4.5 Description of mould growth

Mould growth was scored as described in section 3.3.4.

3.4.6 Statistical analysis

Statistical analysis of the data collected was carried out using MSTAT Software. ANOVA (analysis of variance) and Student Newman Keuls multiple comparison tests were carried out.

3. 5 EFFECT OF CO-FERMENTATION OF CASSAVA WITH COWPEA ON THE TEXTURE OF CASSAVA ROOTS AND VISCOSITY OF CASSAVA FLOUR PASTE

3.5.1 Preparation of spores

Spore suspensions of *Rhizopus stolonifer* (CNF6) and *Neurospora sitophila* (CNF7) were prepared according to the method described by Teran & Owens (1996), as described in section 3.3.2.

3.5.2 Preparation of cassava roots and cowpea

Cassava roots were acquired at a local market in Reading, United Kingdom. The cassava was first washed with water and then with 70% (v/v) ethanol to reduce contamination during the peeling process. The cassava roots were sliced transversally to obtain pieces with a length of 10 cm. Each piece was then sliced twice longitudinally to obtain 4 sliced cassava root pieces.

Cowpea grain (NATCO) was acquired at a local market in Reading, United Kingdom, with the following composition per 100g: protein, 22.5 g; carbohydrates, 59.1 g (sugars 5.1g); fat, 1.1g, fibre 12.5 g; and sodium, less than 0.05 g. The cowpea was milled in a coffee grinder

and then sieved to obtain particles less than 1 mm diameter. The cowpea flour was sterilized by autoclaving at 121 °C for 15 min.

3.5.3 Treatments of batches for fermentation

Cassava root pieces were mixed with cowpea flour in the proportion 92:8 (w/w) (cassava:cowpea). To the mixture, sterilized distilled water was added (80 ml per kg of the mixture). Cassava treatments for fermentation were as follows:

1- Cassava, uninoculated

2- Cassava, inoculated with *Rhizopus stolonifer* (CNF6)

3- Cassava, inoculated with *Neurospora sitophila* (CNF7)

4- Cassava + cowpea, uninoculated

5- Cassava + cowpea, inoculated with *Rhizopus stolonifer* (CNF6)

6- Cassava + cowpea, inoculated with *Neurospora sitophila* (CNF7)

The treatments were carried out in duplicate. Samples were placed into different sterilized plastic containers. For inoculation, the pieces in each container (500 g) were sprayed with mould spore suspension (10^6 spores ml^{-1}) (2.5 ml) using a sprayer. The containers were covered with sterilized tinfoil. The samples were incubated up to 72 hours at 30° C.

3.5.4 Description of mould growth

Mould growth was scored as described in section 3.3.4.

3.5.5 Texture analysis of cassava roots during fermentation

Hardness of the cassava roots was determined as described in section 3.3.5.

3.5.6 Determination of viscosity of cassava flour by "Hagberg Falling Number"

Viscosity of the cassava flour samples was evaluated using the Hagberg Falling Number, which is defined as the time in seconds required to stir and allow the stirrer to fall a measured distance through a hot aqueous flour gel undergoing liquefaction (AACC, 1969). This method is mostly used to measure the effect of ∝-amylase activity on the viscosity of dough made from wheat flour. The flour samples (4 g) were suspended in water in a test tube (25 ml). The test tube with the suspension was placed on the Falling Number equipment (Perten, V 1500). The suspension was stirred under standardised conditions, according to the manufacturer, whilst being heated in a boiling water bath. The starch gelatinises under these conditions to form a paste. If amylase activity is low, the starch will gelatinise to form a relatively viscous paste. If amylase activity is high, however, the paste will be less viscous.

For wheat flour with about 14 % moisture, the method recommends the use of 7 g of flour in 25 ml of distilled water. In this work, the fermented cassava roots were dried in an oven at 50 °C with forced air- circulation, milled and sieved through a sieve of mesh size 210 μm. The moisture content of the flour after 3 days was 8 ±0.6 %. For the 'Falling Number' test, samples of cassava flour (4 g) were suspended in distilled water (25 ml).

3.5.7 Statistical analysis

Statistical analysis of the data collected was carried out using MSTAT Software. ANOVA (analysis of variance) and Student Newman Keuls multiple comparison test were carried out.

3.6 SENSORY EVALUATION OF PASTE (*KARAKATA*) PREPARED USING FLOUR FROM CASSAVA ROOTS CO-FERMENTED WITH COWPEA

3.6.1 Samples used for sensory evaluation

Sensory properties of 8 samples of *karakata* made from cassava flour prepared in the laboratory and 3 samples prepared from cassava flour bought from the local market in Nampula city, Mozambique, were evaluated. The flour samples from the laboratory were prepared as described in section 3.5.3 using cassava roots of the *Fernando po* variety harvested in Amantongas area, Gondola District, Manica Province, Mozambique. Laboratory prepared flour samples were prepared from cassava roots fermented for 72 hours. *Karakata* samples that were evaluated were made from flours prepared as follows:

1. Cassava flour, unfermented
2. Cassava flour, fermented with *R. stolonifer* as inoculum
3. Cassava flour, fermented with *N. sitophila* as inoculum
4. Cassava flour, fermented with a mixture of *R. stolonifer* and *N. Sitophila*, in equal proportions, as inoculum
5. Cassava flour supplemented with cowpea, unfermented
6. Cassava flour, supplemented with cowpea and fermented with *R. stolonifer* as inoculum
7. Cassava flour, supplemented with cowpea and fermented with *N. sitophila* as inoculum
8. Cassava flour, supplemented with cowpea and fermented with a mixture of *R. stolonifer* and *N. Sitophila*, in equal proportions, as inoculum
9. Cassava flour, unfermented from local market
10. Cassava flour, fermented, from local market 1
11. Cassava flour, fermented, from local market 2

3.6.2 Preparation of cassava flour paste (*karakata*)

Cassava flour paste in the form of a thick porridge, *karakata*, was prepared as follows: each cassava flour sample (60 g) was placed into boiling distilled water (150 ml), stirred and left to boil for about 3 minutes until a thick paste was formed. The *karakata* was immediately served in plates.

3.6.3 Sensory evaluation of *karakata*

The method used for the sensory tests was "**Attribute difference tests**" **Hedonic ratings** (Monteiro, 1997). The attributes tested in the different samples were appearance, odour, taste and texture. The taste panel was composed of 10 semi-trained panellists who were occasional consumers of the product in Nampula Province. To evaluate the acceptability of each of the taste attributes, scores were assigned as shown in table 3.1.

Table 3.1 A table showing assigned scores for various sensory attributes of *karakata*

Appearance, Odour and Texture	**Taste**	**Score number**
Very good	Like very much	9
Good	Like	8
Moderately good	Like moderately	7
Slightly good	Slightly like	6
Not good not bad	Don't like and don't dislike	5
Slightly bad	Slightly dislike	4
Moderately bad	Moderately dislike	3
Bad	Dislike	2
Very bad	Dislike very much	1

An evaluation was carried out to determine the flavour characteristics of the *karakata* samples ranging from sweet, bitter, neither sweet nor bitter to sour. The taste was determined by the 10 panellists. The panellists ate soup prepared from fish boiled in distilled water and drank mineral water between tasting of each sample.

3.6.4 Determination of cyanogenic potential

The cyanogenic potential was determined on all the cassava flour samples using picrate paper kits as described by Bradbury *et al.* (1999). Cassava flour samples (100 mg) were added to small, flat bottomed plastic vials (25 mm diameter, 50 mm high) followed by phosphate buffer (0.1 mol l^{-1}) at pH 6 (0.5 ml), enzyme solution prepared as described in section 3.1.3.2 (100 µl) and picrate paper prepared according to Bradbury *et al.* (1999). The plastic vials were immediately closed with screw caps and incubated at 30 °C for 16 hours. The picrate paper was removed from the vials, immersed in distilled water (5 ml) and shaken by a vortex mixer (Stuart) for about 20 seconds then left to stand for 30 min. After standing the absorbance of the solution was measured at 510 nm using a spectrophotometer (Spectronic 20 Genesys) against a blank consisting of phosphate buffer. The cyanogenic potential, expressed as mg HCN per kg of sample, was obtained by multiplying the absorbance by 396 (Bradbury *et al.*, 1999). A filter paper disc (21 mm diameter) loaded with 50 mg HCN kg^{-1} was used as standard. For fresh cassava roots the procedure was similar, but without addition of enzyme solution.

3.6.5 Statistical analysis

Statistical analysis of the data collected was carried out using ANOVA (analysis of variance). Tukey's multiple comparisons test was used to compare specific attributes for the various samples.

4.0 RESULTS

4.1 CYANOGENIC POTENTIAL OF CASSAVA FLOUR COLLECTED FROM SELECTED DISTRICTS OF NAMPULA PROVINCE, MOZAMBIQUE

4.1.1 Comparison of processing methods in terms of cyanogenic potential

The mean values of the cyanogenic potential of the fermented and unfermented samples are shown in (Table 4.1). In general, the cyanogenic potential value obtained was higher for the unfermented samples than for the fermented samples. The mean value of the total cyanogenic content for the fermented samples was 35 ± 30 mg HCN kg^{-1} while that for the non-fermented samples was 65 ± 60 mg HCN kg^{-1}. The mean for the total unfermented samples was significantly greater than that of the fermented samples ($p < 0.001$).

The distribution pattern of cyanogenic content for all cassava samples is shown in (Figure 4.1). Twelve percent of the collected samples had more than 100 mg HCN kg^{-1} of cyanogenic potential. Two samples had more than 200 mg HCN kg^{-1} cyanogenic potential. The distribution pattern of the total cyanogenic content for the cassava samples processed by a combination of fermentation followed by sun-drying is shown in Figure 4.2, and that for samples processed by sun-drying alone is shown in Figure 4.3. Most of the flour samples processed by a combination of fermentation followed by sun-drying had a cyanogenic potential value of less than 60 mg HCN kg^{-1}.

Table 4.1. Mean cyanogen contents (mg HCN/kg) of samples of cassava flour from different districts after processing. Uncertainties are shown as standard deviations

	District				
Processing Method	**Memba**	**Monapo**	**Mogincual**	**Mogovolas**	**Total**
Fermented and unfermented samples	90 ± 60	26 ± 20	35 ± 20	26 ± 30	44 ± 50
Number of samples analysed	28	30	27	23	108
Fermented samples	60 ± 50	20 ± 20	36 ± 30	21 ± 20	34 ± 30
Number of samples analysed	17	18	20	16	71
Unfermented samples	130 ± 50	36 ± 30	30 ± 20	37 ± 40	64 ± 60
Number of samples analysed	11	12	7	7	37

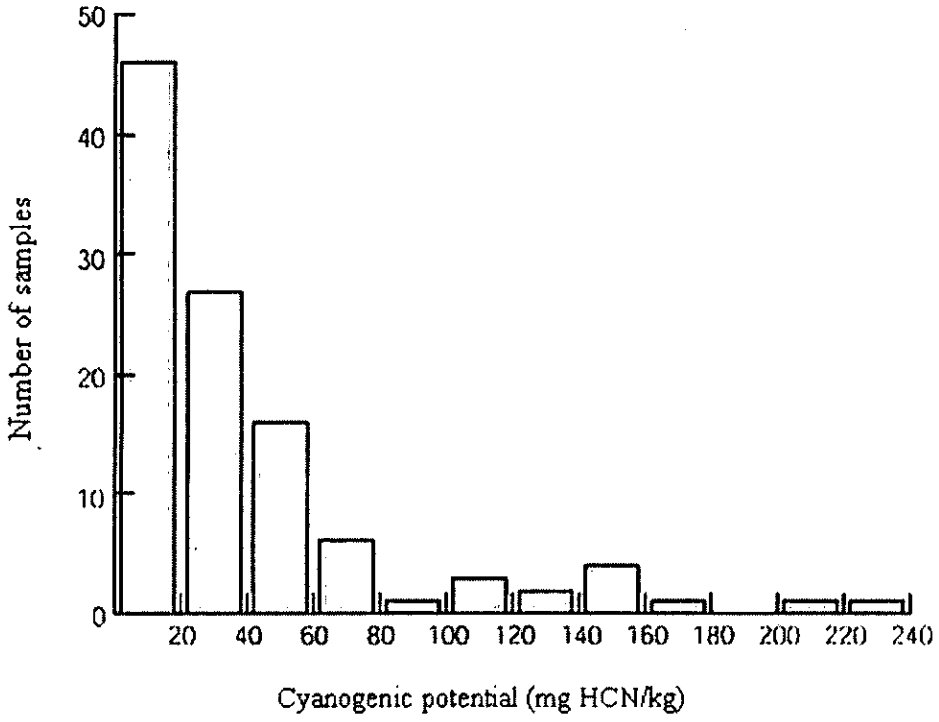

Figure 4.1. Distribution of the cyanogenic potentials of all samples of cassava flour

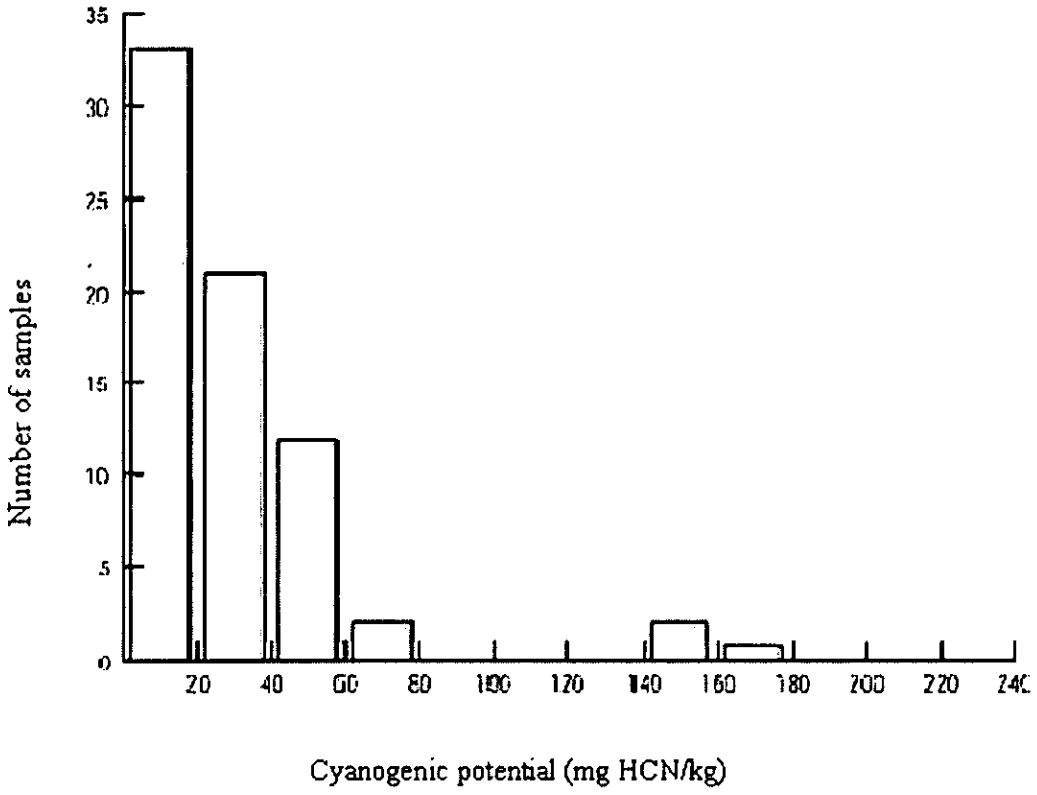

Figure 4.2 Distribution of the cyanogenic potentials of samples of cassava flour processed by a combination of fermentation followed by sun-drying

For the cassava flour samples processed by sun-drying only, a significant proportion (24.3%) had more than 100 mg HCN kg^{-1} of cyanogenic potential. The distribution pattern for these unfermented cassava flour samples showed multiple peaks. In general, the cyanogenic potential values of cassava flours produced by a combination of fermentation and sun-drying were lower than those obtained by sun-drying alone.

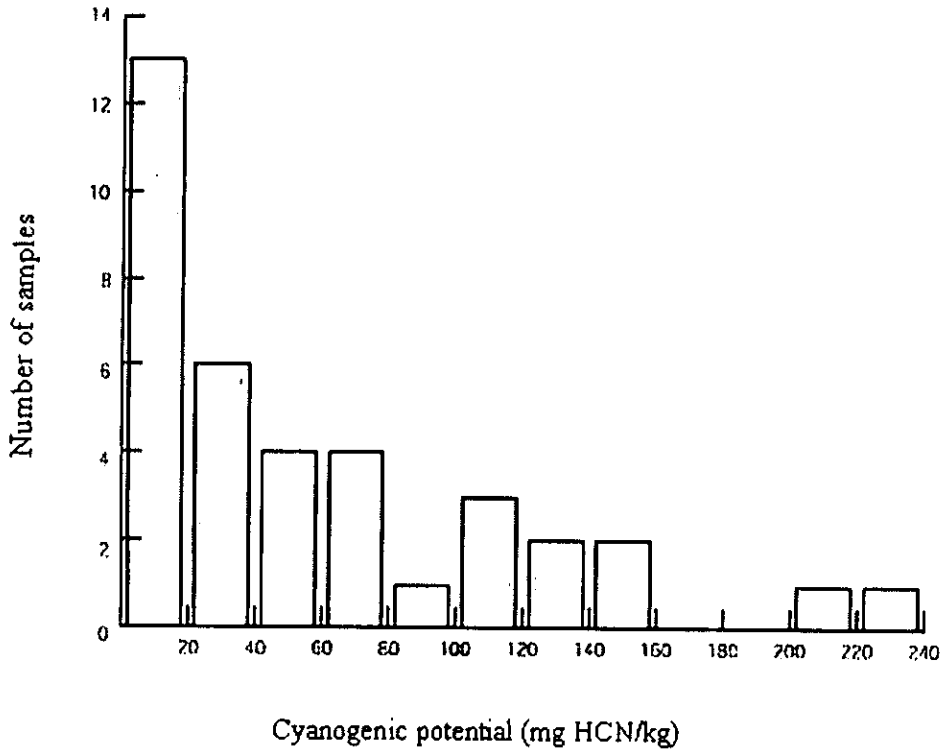

Figure 4.3 Distribution of cyanogenic potentials of samples of cassava flour processed by sun-drying alone

4.1.2 Comparison of the cyanogenic potential of flour samples from four different locations

The four locations all grew mostly bitter varieties for fermented and unfermented flour production, while the roots from sweet varieties were eaten fresh, boiled and for unfermented cassava flour. The mean cyanogenic potential values of the flour samples collected from the four different locations are shown in Table 4.1

At location 1, in Memba district, the mean cyanogenic potential value for the unfermented samples was significantly greater than that for the fermented samples ($p < 0.001$). The distribution pattern for the flour samples obtained for this district is shown in Figure 4.4. Sixteen flours were produced from *Mulapa*, which is a bitter variety, while seven flours were from *Karita*, another bitter variety. Two flour samples were from each of the sweet varieties *Oladha* and *Maria*. The two sweet varieties gave two flours samples with the lowest cyanogenic potential for this district. Two flour samples had a cyanogenic potential value above 200 mg HCN kg^{-1} and eleven flour samples had a cyanogenic potential value above 100 mg HCN kg^{-1} in this district. Three fermented flour samples had a cyanogenic potential value above 100 mg HCN kg^{-1}. None of the flour samples collected from this district had cyanogenic potential values below 15 mg HCN kg^{-1}. In Memba district, there were serious problems with viral infection of the roots at the time the flours were collected.

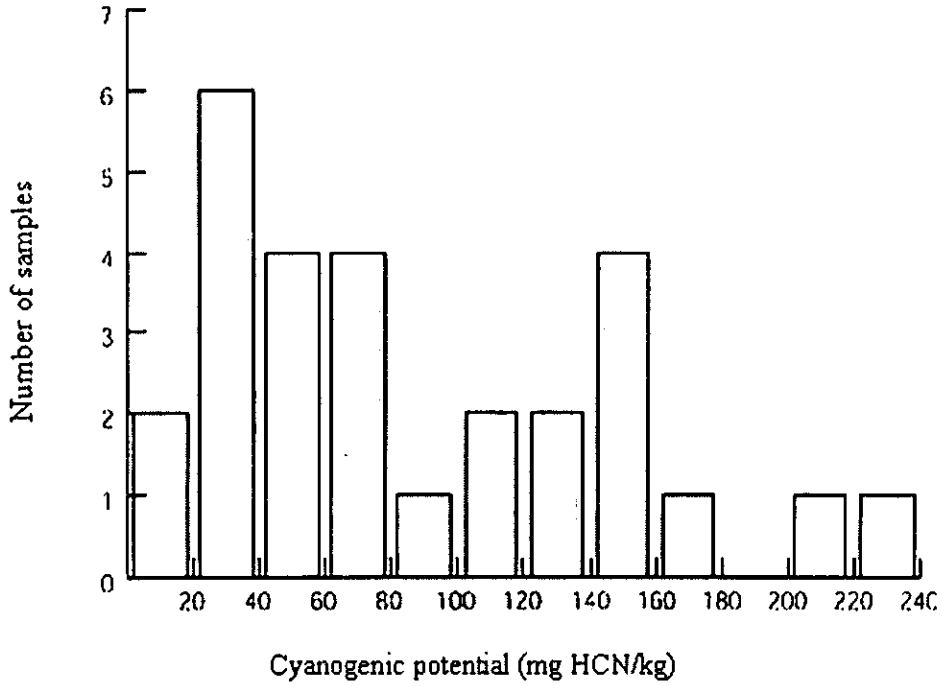

Figure 4.4 Distribution of the cyanogenic potentials of cassava flour samples from Acordo de Lusaka, Memba District

At location 2 in Monapo district, the mean cyanogenic potential value for the unfermented samples was significantly greater than that for the fermented samples ($p < 0.05$). The distribution pattern of flours collected from homes in Monapo District is shown in Figure 4.5. In this district, the bitter variety grown for flour processing in this location was *Mulapa*, which was the variety used to produce eighteen of the flours collected. The flour samples collected included two samples from each of the sweet varieties *Namalithi* and *Guerra*. Four flour samples were made from *Ntheda*, a bitter variety. One flour sample collected at Monapo district was made from mixing the following bitter varieties: *Nivalapua*, *Nassuruma*, *Mulapa*, *Nlobia* and *Gogoro*. There were no flour samples with cyanogenic potential values above

100 mg HCN kg^{-1} collected in this district. Eighteen flour samples had cyanogenic potential values of less than 20 mg HCN kg^{-1}.

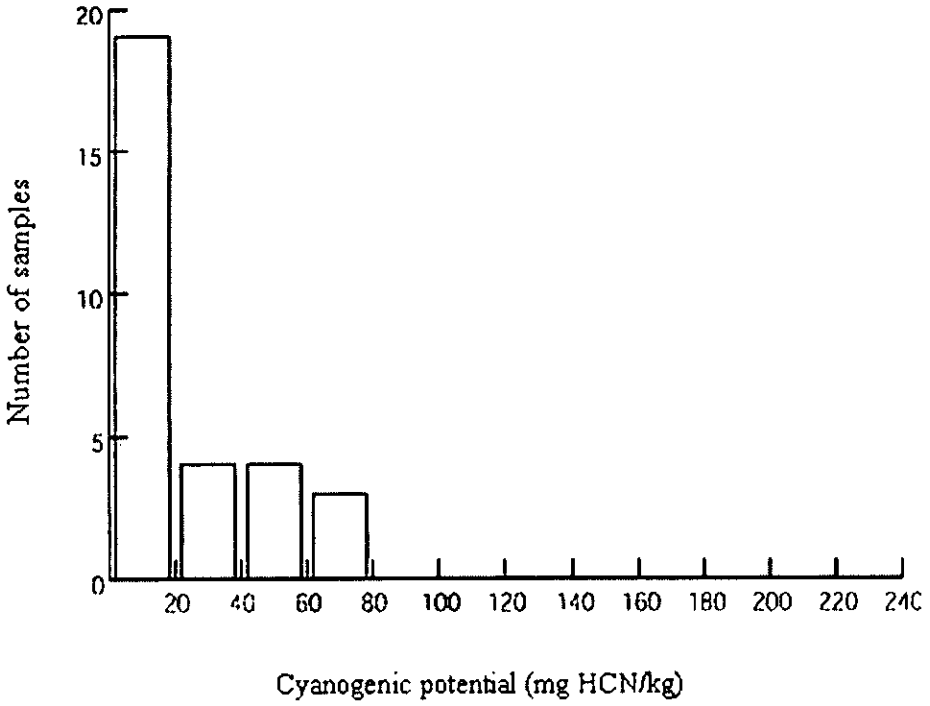

Figure 4.5 Distribution of the cyanogenic potentials of cassava flour samples from Niyaro village, Monapo District

In Mogincual district, there was no significant difference in the mean cyanogenic potential value between unfermented and fermented samples ($p > 0.05$). The number of flour samples collected at location 3 in Mogincual district, was twenty-seven. Of these flour samples, twenty-two were processed from the variety *Tomo*. Two of the flours were made from the sweet variety, *Niacy*, and the varieties *Nivalapua* and *Uhope* accounted for one flour sample each. The distribution pattern of flours collected from homes in Mogincual District is shown in Figure 4.6. There was one unfermented flour samples with cyanogenic potential values above 100 mg HCN kg^{-1}. The number of unfermented flour samples was seven and four of them were

from sweet varieties. Only one flour sample, from the sweet variety, *Uhope*, had a cyanogenic potential below 10 mg HCN kg^{-1}.

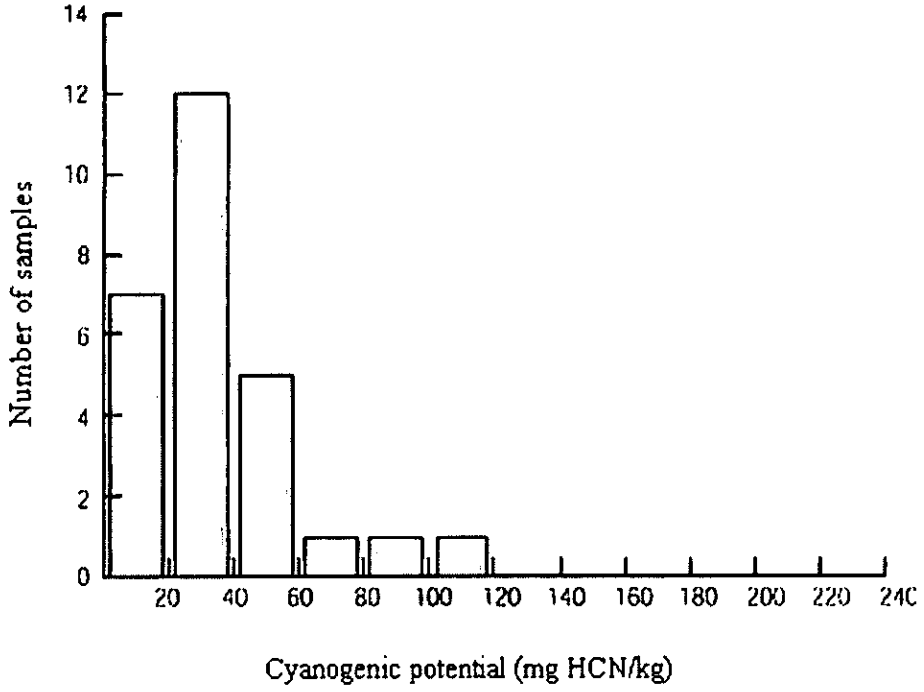

Figure 4.6 Distribution of the cyanogenic potentials of cassava flour samples from Quixaxe village, Mogincual District

At location 4 in Mogovolas district, the mean cyanogenic potential value for the unfermented samples was significantly greater than that for the fermented samples ($p < 0.05$). In Mogovolas, nineteen samples were from *Tomo*, and three samples from *Nivalapa*, both bitter varieties. Only one sample was from sweet variety *Maria*.

The distribution pattern of flours collected from homes in Mogovolas District is shown in Figure 4.7. The total number of flour samples collected was twenty-three and eleven flour

samples had cyanogenic potential values below 10 mg HCN kg^{-1}. One unfermented white flour sample had a cyanogenic potential value above 100 mg HCN kg^{-1}.

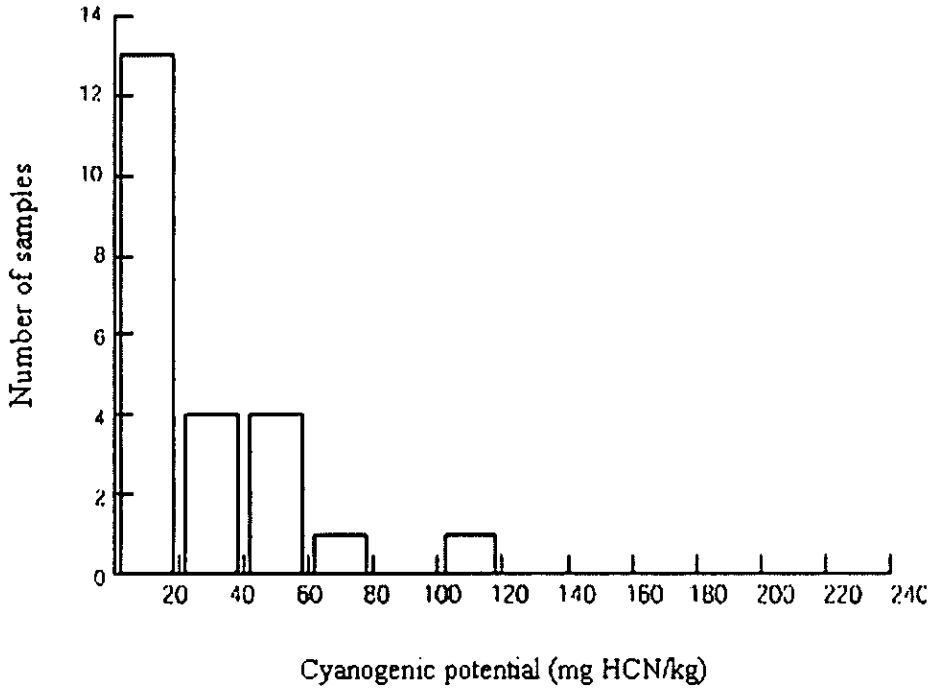

Figure 4.7 Distribution of the cyanogenic potentials of cassava flour samples from Naconha village , Mogovolas District

For the fermented samples, the mean values of the cyanogenic potentials were significantly different for the following districts: Memba-Monapo, Memba-Mogovolas, Memba Mogincual and Monapo-Mogincual, with p – values of 0.0025, 0.0069, 0.014 and 0.0205, respectively.

4.2 MICROBIAL AND BIOCHEMICAL CHARACTERISTICS OF CASSAVA HEAP FERMENTATION

4.2.1 Traditional method of heap fermentation in Nampula Province, Mozambique

Households 1 and 2 grew *Nakamula* variety while household 3 grew *Haheta* variety, which are both bitter cassava varieties. Nine month old (period after planting) cassava roots were used. The roots had been attacked by the disease, locally called cassava root rot, affecting 10 to 20% of the harvested cassava roots. At the time of the study, the disease was currently a problem in cassava roots in the region.

The method used for heap fermentation was generally characterized by peeling of the cassava roots longitudinally using a knife to remove the cortex. During the peeling, part of the storage parenchyma was also removed, which caused some damage on the surface of the pulp. The ends of each root were removed, and roots longer than 50 cm were divided by cutting transversely. The cassava roots were immediately piled into heaps. In all 3 households, the amount of roots was approximately the same, about a volume of approximately 50 litres.

The heaps in the three households were placed under the shade, but in different locations. In household 1, the heap was under the roof of the house, but outside, and the cassava was covered with cassava peel and dry leaves. In household 2, the heap was under a tree close to the trunk and the roots were covered with cassava peel and cashew nut tree leaves, and in household 3, the heap was inside the house veranda to prevent the goats from disturbing the heaps. The cassava roots were covered with cassava peel and other tree leaves.

4.2.2 Visual changes, temperature and pH in the heaps

No moulds were observed on the surfaces of cassava roots on the first and second days of fermentation but the surfaces of the roots became slimy, probably due to bacterial and/or yeast

growth. Moulds appeared on the roots on the third day, starting with areas of root damage. On the fourth day, the moulds had almost covered all the roots, which had become soft when touched and pressed. At this stage the cassava roots were ready to be dried in the sun.

Condensation was observed on the roots and the leaves during fermentation. At the beginning of fermentation, the inside and outside temperatures of the heaps were the same in the morning. However, by midday, inside temperatures were lower than the outside temperatures (Figures 4.8 and 4.9).

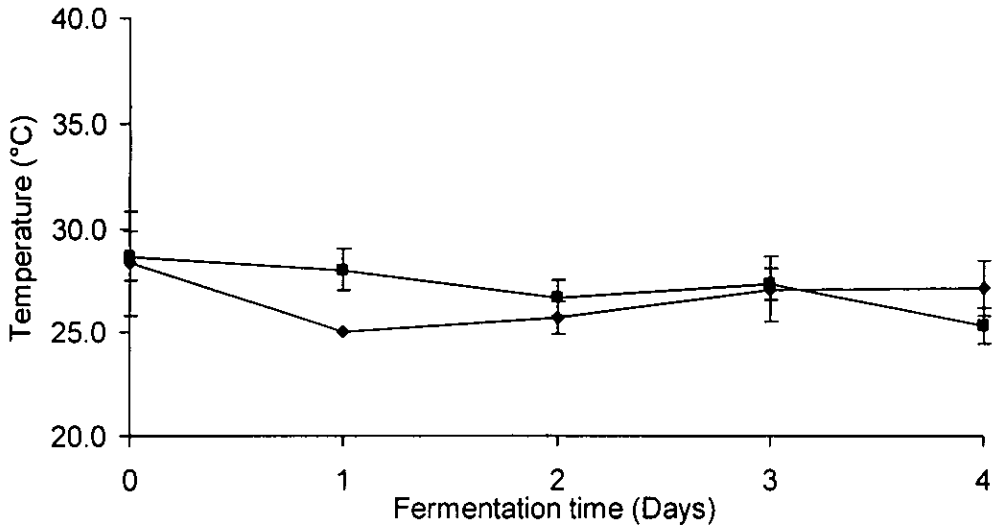

Figure 4.8. Temperatures inside (—◆—) and outside (—■—) the heaps during traditional fermentation, measured in the morning (7 to 8 o'clock a.m.). Error bars represent the standard deviation of 3 independent heaps

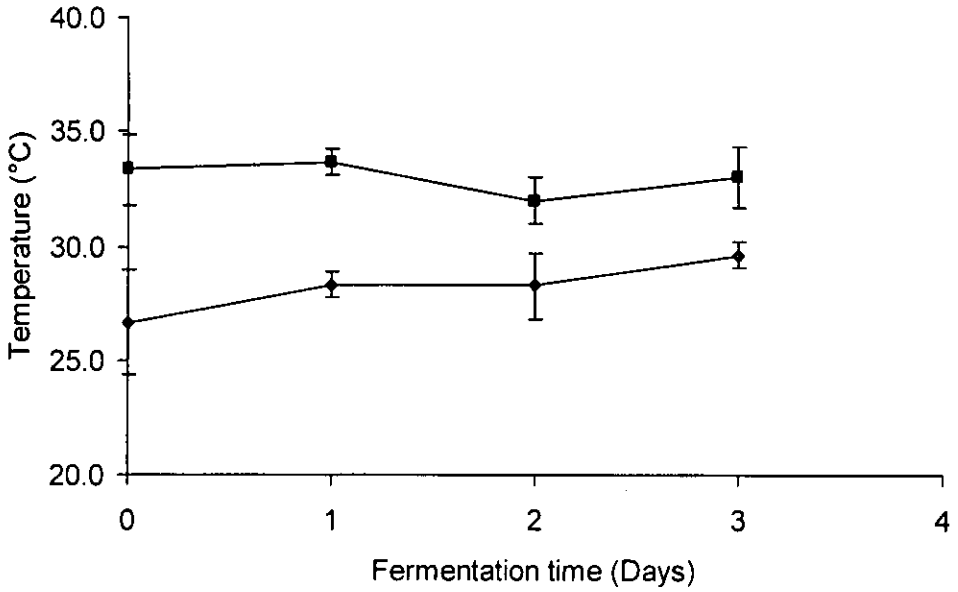

Figure 4.9 Temperatures inside (—◆—) and outside (—■—) the heaps during traditional fermentation, measured in the afternoon (1 to 2 o'clock p.m.). Error bars represent the standard deviation of 3 independent heaps

The pH of the cassava roots decreased during fermentation (Table 4.2), probably due to lactic acid production by lactic acid bacteria.

4.2.3 Microbial analysis

It was difficult to determine reliable counts of yeast and moulds due to extensive mould growth on the plates. Total mesophilic bacteria increased from 10^5 to 10^8 cfu/g and lactic acid bacteria increased from 10^3 to 10^6 cfu/g during the fermentation (Table 4.2).

Table 4.2 Table of pH, total mesophilic bacteria and lactic acid bacteria counts in fermented cassava roots, and cyanogenic potential and crude protein in dried fermented cassava chips after different periods of fermentation

	Fermentation time (days)				
	0	**1**	**2**	**3**	**4**
pH of cassava roots (n=3)	6.1 ± 0.1	6.1 ± 0.1	6.0 ± 0.4	5.6 ± 0.5	5.6 ± 0.6
Total mesophilic bacteria in cassava roots (log cfu/g) (n=3)	5.1 ± 0.2	6.9 ± 0.4	7.3 ± 0.2	7.6 ± 0.1	7.6 ± 0.0
Total lactic acid bacteria in cassava roots (log cfu/g) (n=3)	3.3 ± 0.9	4.2 ± 0.2	5.2 ± 0.5	5.7 ± 0.1	5.8 ± 0.1
Crude protein concentration in dried cassava roots (% w/w) (n=3)	1.3 ± 0.1	1.4 ± 0.1	1.5 ± 0.3	1.8 ± 0.2	1.6 ± 0.1
Cyanogenic potential in dried and fermented cassava roots (mg HCN/kg) (n=3)	158 ± 20	117± 25	30 ± 13	23 ± 11	17 ± 6

Average and standard deviation from 3 independent heaps

4.2.4 Identification of microorganisms

4.2.4.1 Lactic acid bacteria

Of the 10 bacterial isolates from heap fermented cassava roots, 4 were cocci and 6 were rods. Three strains were catalase positive and 7 were negative (Table 4.3). Of the 7 catalase negative strains, 6 were identified as lactic acid bacteria: *Leuconostoc pseudomesenteroides* (CNB1) *Leuconostoc pseudomesenteroides* (CNB4), *Leuconostoc mesenteroides* (CNB6), *Enterococcus faecium* (CNB7) and *Weissella cibaria* (CNB9) from fermented cassava. *Leuconostoc mesenteroides* (CNB10) was also isolated from dried cassava flour.

Table 4.3 Summary of the characteristics of bacteria isolated from heap fermented cassava

Bacterial isolate code	Sample origin	Cell morphology	Final pH in APT broth	Catalase Test	Strain Identification
CNB1	3 day heap fermented cassava roots from location 2	Rods, single and paired	4.67	-	*Leuconostoc pseudomesenteroides*
CNB2	3 day heap fermented cassava roots from location 1	Cocci, single and paired	4.52	+	ND
CNB3	3 day heap fermented cassava roots from location 1	Rods, single and paired	6.36	+	ND
CNB4	3 day heap fermented cassava roots from location 1	Rods, single and paired	4.65	-	*Leuconostoc pseudomesenteroides*
CNB5	3 day heap fermented cassava roots from location 1	Rods, single and paired	4.80	-	ND
CNB6	2 day heap fermented cassava roots from location 1	Cocci, single and paired	4.74	-	*Leuconostoc mesenteroides*
CNB7	2 day heap fermented cassava roots from location 1	Cocci, single and paired	4.05	-	*Enterococcus faecium*
CNB8	2 day heap fermented cassava roots from location 1	Rods, single and paired	6.13	+	ND
CNB9	dried, fermented cassava roots from location 2	Cocci, single and paired	4.70	-	*Leuconostoc mesenteroides*
CNB10	1 day heap fermented cassava roots from location 3	Rods, single and paired	4.87	-	*Weissella cibaria*

+ positive; - negative, ND- Not identified

4.2.4.2 Moulds

Moulds were observed in 2 to 3 day fermented cassava roots and in dried fermented cassava. A black mould was dominant and grew in the medium used. The orange moulds were identified as *Neurospora sitophila* and the black moulds as *Rhizopus stolonifer* (Table 4.4).

Table 4.4 Summary of the characteristics of moulds isolated from heap fermented cassava roots

Mould isolate code	Sample origin	Mould growth characteristics and mycelial morphology	Strain Identification
CNF1	Dry cassava chips from location 1	Spread rapidly on the medium starting with white mycelia and within 36 hours turned orange with sporulation. Spherical conidia were observed under the microscope.	*Neurospora sitophila*
CNF2	3 day heap fermented cassava roots from location 2	Small grey green colonies, septate hyphae.	ND
CNF3	3 day heap fermented cassava roots from location 1	Within 12 hours, white mycelia covered whole medium. After 36 hours, mycelia become black mainly at the margin of plate. Sporangiospores with striate walls were observed under the microscope.	*Rhizopus stolonifer*
CNF4	3 day heap fermented cassava roots from location 3	Within 12 hours, white mycelia covered whole medium. After 36 hours, mycelia become black mainly at the margin of plate. Sporangiospores with striate walls were observed under the microscope.	*Rhizopus stolonifer*
CNF6	Dried cassava chips from location 1	Within 12 hours, white mycelia covered whole medium. After 36 hours, mycelia become black mainly at the margin of plate. Sporangiospores with striate walls were observed under the microscope.	*Rhizopus stolonifer*
CNF7	3 day heap fermented cassava roots from location 2	Spread rapidly on the medium starting with white mycelia and within 36 hours turned orange with sporulation. Spherical conidia were observed under the microscope.	*Neurospora sitophila*

ND – not identified

4.2.5 Cyanogenic potential and crude protein content of dry cassava chips

The cyanogenic potential in fresh cassava roots were 250 ± 22, 262 ± 9 and 266 ± 20 mg HCN/kg fresh weight basis for households 1, 2 and 3 respectively, resulting an average of 259 ± 9 mg HCN/kg fresh weight basis. Sun drying alone resulted in a reduction of total cyanogen contents by 40%. Fermentation of cassava roots for 1, 2, 3 and 4 days followed by sun drying resulted in a reduction of total cyanogens by 55, 88, 91 and 93% respectively. Cyanogenic potential of cassava chips, heap fermented for 4 days and sun dried were 9 - 14% of that in sun dried unfermented chips which had an average value of 158 ± 20 mg HCN/kg (Table 4.2). The average protein content in dried cassava roots slightly increased from 1.3 ± 0.1 % to 1.8 ± 0.2 % (w/w) after 3 days of fermentation (Table 4.2).

4.3 LABORATORY SIMULATION OF CASSAVA HEAP FERMENTATION USING ISOLATED MOULDS

4.3.1 Description of mould growth on different pieces of cassava root

After 24 hours of solid-state fermentation, the surface of all the cassava pieces changed from white to brown. On roots inoculated with both *Neurospora sitophila* and *Rhizopus stolonifer*, white mycelia started to appear. Roots inoculated with *Neurospora sitophila* had more mycelia than roots inoculated with *Rhizopus stolonifer* at 24 hours of fermentation (Table 4.5).

After 48 hours, there was appreciable mycelial growth on longitudinally sliced cassava root pieces, both 8 cm long and 4 cm long. In these pieces, *Neurospora sitophila* mycelia was more than on roots inoculated with *Rhizopus stolonifer*.

In the cassava pieces which were not longitudinally sliced, both 8 cm long and 4 cm long, mycelial growth from both *Neurospora sitophila* and *Rhizopus stolonifer* was intensive on

the transversal cut surface. In the pieces of cassava roots inoculated with *Rhizopus stolonifer*, there was no mycelia on the surface of cassava pieces where cassava peel was removed.

Intense growth of moulds was observed after 72 hours of incubation in the following cassava roots pieces: 8 cm long and 4 cm, long both sliced longitudinally, and 4 cm long, not sliced longitudinally. The moulds covered almost the roots and a lot of spore production was observed. In the uninoculated cassava roots, no fungal growth was observed under controlled condition.

There was no bacterial growth detected in thioglycollate broth from the cassava samples during the incubation.

Table 4.5 Mould growth on different slices of cassava roots during fermentation at 30° C

Treatment	**Fermentation time (hours)**			
	0	**24**	**48**	**72**
Uninoculated cassava roots (control)	0	0	0	0
Inoculated with *Rhizopus stolonifer*:				
Cassava roots, 8 cm long	0	1	1	2
Cassava roots, 8 cm long, sliced longitudinally once	0	2	3	3
Cassava roots, 4 cm long	0	2	2	3
Cassava roots, 4 cm long, sliced longitudinally once	0	2	3	4
Inoculated with *Neurospora sitophila*:				
Cassava roots, 8 cm long	0	1	2	3
Cassava roots, 8 cm long, sliced longitudinally once	0	2	3	4
Cassava roots, 4 cm long	0	1	3	4
Cassava roots, 4 cm long, sliced longitudinally once	0	2	4	4

0- No growth (no mycelia), **1**- Slight growth (less than 1/4 of the roots covered with mycelia), **2**- Moderate growth (more than 1/4 and less than 1/2 of the roots covered with mycelia), **3**- Growth (more than 1/2 of the roots covered with mycelia), **4**- Good growth (all roots covered with mycelia)

4.3.2 Determination of cassava softness and pH

Cassava roots inoculated with *Rhizopus stolonifer* were softer than roots inoculated with *Neurospora sitophila* (Table 4.6). The uninoculated control remained hard.

Comparing the softness between the different pieces of cassava roots inoculated with moulds, 8 cm long non-longitudinally sliced pieces were less soft than all other inoculated pieces during the fermentation. Longitudinally sliced roots, 4 cm long, were the softest after 72 hours of fermentation followed by longitudinally sliced roots, 8 cm long, then unsliced roots, 4 cm long. Longitudinally unsliced cassava roots, both 4 and 8 cm long were very soft on the transversal surface.

Table 4.6 Texture of different slices of unfermented and fermented cassava roots

Treatment	Hardness of cassava roots (g force of peak compression force)	
	Unfermented	Fermented up to 72 hours
Uninoculated cassava roots (control)	2400 ± 300	2300 ± 100
Inoculated with *Rhizopus stolonifer*:		
Cassava roots, 8 cm long	2500 ± 200	1200 ± 50
Cassava roots, 8 cm long, sliced longitudinally once	2100 ± 200	150 ± 20
Cassava roots, 4 cm long	2300 ± 200	250 ± 200
Cassava roots, 4 cm long, sliced longitudinally once	2200 ± 400	130 ± 60
Inoculated with *Neurospora sitophila*:		
Cassava roots, 8 cm long	2200 ± 50	1500 ± 160
Cassava roots, 8 cm long, sliced longitudinally once	2100 ± 100	1600 ± 100
Cassava roots, 4 cm long	2100 ± 231	1500 ± 400
Cassava roots, 4 cm long, sliced longitudinally once	2200 ± 217	1400 ± 60

Average and standard deviation from 3 independent samples

The average pH of all the fermented cassava samples decreased from 6.5 ± 0 to 5.8 ± 0.1 after 72 hours of incubation (Table 4.7).

Table 4.7 pH changes during the fermentation of batches of cassava at 30 °C

Treatment	Fermentation time (hours)			
	0	**24**	**48**	**72**
Uninoculated cassava roots (control)	6.5	6.3 ± 0.2	6.2 ± 0.1	6.2 ± 0.1
Inoculated with *Rhizopus stolonifer*:				
Cassava roots, 8 cm long	6.4 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	5.8 ± 0.1
Cassava roots, 8 cm long, sliced longitudinally once	6.5	6.0 ± 0.2	6.0 ± 0.2	6.0 ± 0.1
Cassava roots, 4 cm long	6.4	6.0 ± 0.1	6.0 ± 0.1	5.8 ± 0.1
Cassava roots, 4 cm long, sliced longitudinally once	6.5 ± 0.1	6.1	5.9 ± 0.3	5.7 ± 0.3
Inoculated with *Neurospora sitophila*:				
Cassava roots, 8 cm long	6.5 ± 0.1	6.0 ± 0.1	6.1	5.9 ± 0.1
Cassava roots, 8 cm long, sliced longitudinally once	6.5	6.2 ± 0.3	6.0 ± 0.1	5.9 ± 0.2
Cassava roots, 4 cm long	6.5	6.1 ± 0.1	6.0 ± 0.2	5.8 ± 0.3
Cassava roots, 4 cm long, sliced longitudinally once	6.5	6.2 ± 0.1	6.1	5.8

Average and standard deviation from 3 independent samples

4.4 PROTEIN ENRICHMENT OF CASSAVA BY CO-FERMENTATION WITH COWPEA

4.4.1 Description of mould growth

In samples of cassava supplemented with cowpea at the proportions 96:4 and 92:8 (w/w) moulds grew with mycelia covering all the roots after 48 hours (Table 4.8). For cassava roots

without cowpea supplementation, inoculated with *Rhizopus stolonifer* (*R. stolonifer*), mycelia covered more than ½ of the roots after 72 hours. In cassava roots supplemented with cowpea at the proportion 88:12 (w/w) the growth rate of mycelia reduced after 48 hours, and the cassava roots were not fully covered after 72 hours (Table 4.8). In uninoculated cassava samples, no mould growth was observed. The surface of uninoculated cassava samples appeared dry.

During the fermentation, there was an unpleasant smell in the inoculated samples, with the smell being more intensive in samples with cowpea. In the sun dried fermented cassava roots, however, there was no unpleasant smell. Inoculated cassava samples without cowpea were brown in colour, while samples with cowpea were dark brown.

TABLE 4.8 Growth of *Rhizopus stolonifer* (*R. stolonifer*) during fermentation of cassava and cassava + cowpea at 30° C

Treatment	**Fermentation time (hours)**			
	0	**24**	**48**	**72**
Uninoculated samples	0	0	0	0
Cassava inoculated with *R. stolonifer*	0	1	2	3
Cassava + cowpea (96:4) + *R. stolonifer*	0	2	4	4
Cassava + cowpea (92:8) + *R. stolonifer*	0	2	4	4
Cassava + cowpea (88:12) + *R. stolonifer*	0	2	3	3

0- No growth (no mycelia), **1**- Slight growth (less than 1/4 of the roots covered with mycelia), **2**- Moderate growth (more than 1/4 and less than 1/2 of the roots covered with mycelia), **3**- Growth (more than 1/2 of the roots covered with mycelia), **4**- Good growth (all roots covered with mycelia)

4.4.2 pH

There was no significant change in pH ($p > 0.05$) during fermentation to different time periods for all the treatments (Table 4.9).

Table 4.9 pH changes during the fermentation of batches of cassava and cassava + cowpea at 30 °C

Treatment	Fermentation time (hours)			
	0	24	48	72
Cassava, uninoculated	6.5 ± 0.1	6.7 ± 0.2	6.4 ± 0.4	6.5 ± 0.3
Cassava + cowpea (96:4), uninoculated	6.2 ± 0.1	6.5 ± 0.1	6.1 ± 0.1	6.3
Cassava + cowpea (92:8), uninoculated	6.5	6.3	6.4 ± 0.2	6.3 ± 0.1
Cassava + cowpea (88:12), uninoculated	6.1	6.4 ± 0.4	6.2 ± 0.4	6.2 ± 0.1
Cassava alone + *R. stolonifer*	6.0 ± 0.1	6.5 ± 0.3	6.2	6.2
Cassava + cowpea (96:4) + *R. stolonifer*	6.0 ± 0.1	6.6 ± 0.1	6.0 ± 0.4	6.2 ± 0.2
Cassava + cowpea (92:8) + *R. stolonifer*	6.5	6.2 ± 0.2	6.2 ± 0.1	6.3
Cassava + cowpea (88:12) + *R. Stolonifer*	6.4	6.9 ± 0.1	6.8 ± 0.5	6.5 ± 0.3

Average and standard deviation from 2 independent samples

4.4.3 Protein content

Figures 4.10 and 4.11 show respectively the changes in the protein contents of inoculated and uninoculated cassava roots supplemented with varying contents of cowpea with fermentation time. There was no significant difference in total protein contents ($p > 0.05$) between cassava flour from uninoculated samples and samples inoculated with *Rhizopus stolonifer* for the different times of fermentation.

There was a significant increase in the protein content of cassava flour with increase in cowpea flour added to cassava roots ($p < 0.05$). Figure 4.12 shows a comparison of the experimental average protein contents of cassava and cassava:cowpea mixtures inoculated with *Rhizopus stolonifer* and fermented up to 72 hours at 30 °C with the predicted values. In the experimental values, there was a 100% increase in protein content in cassava when cowpea was added in the proportion 96:4. In proportions of 92:8 and 88:12 (cassava:cowpea), protein content increased by 451 and 695% respectively. In the cassava:cowpea proportion of 92:8, the

final protein content in cassava roots flour, about 7.93 ± 0.98 % dry weight basis, is approximately the same of that of maize (Agri-Outreach, 2003).

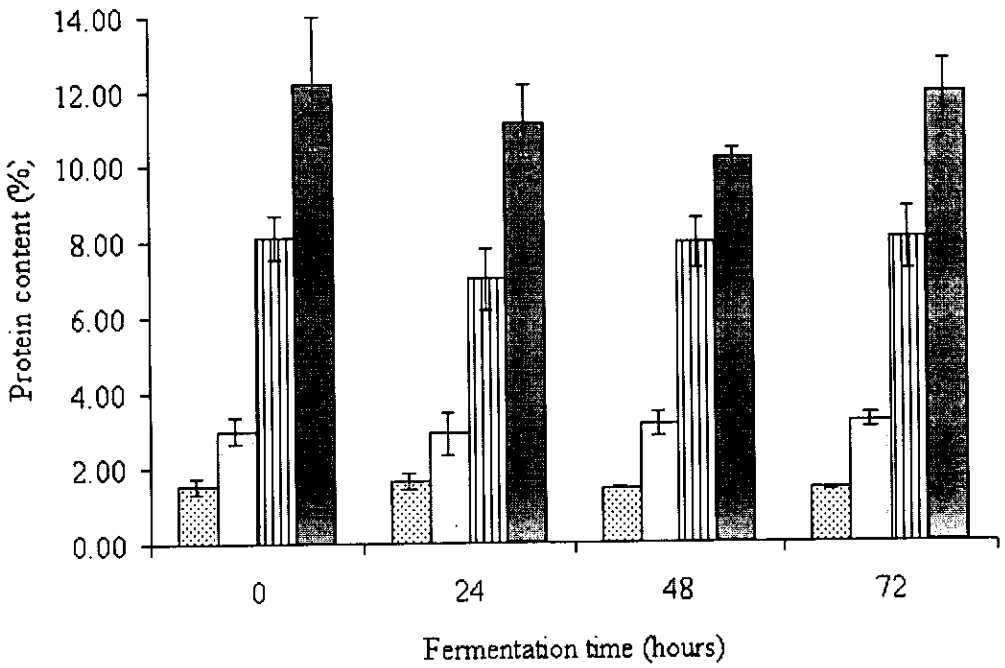

Figure 4.10 Changes in protein content during fermentation of cassava roots inoculated with *Rhizopus stolonifer*. Cassava roots only (▨); Cassava + cowpea, 96:4 (□); Cassava + cowpea, 92:8 (▥); Cassava + cowpea, 88:12 (▤). Error bars represent the standard deviation of 2 independent samples

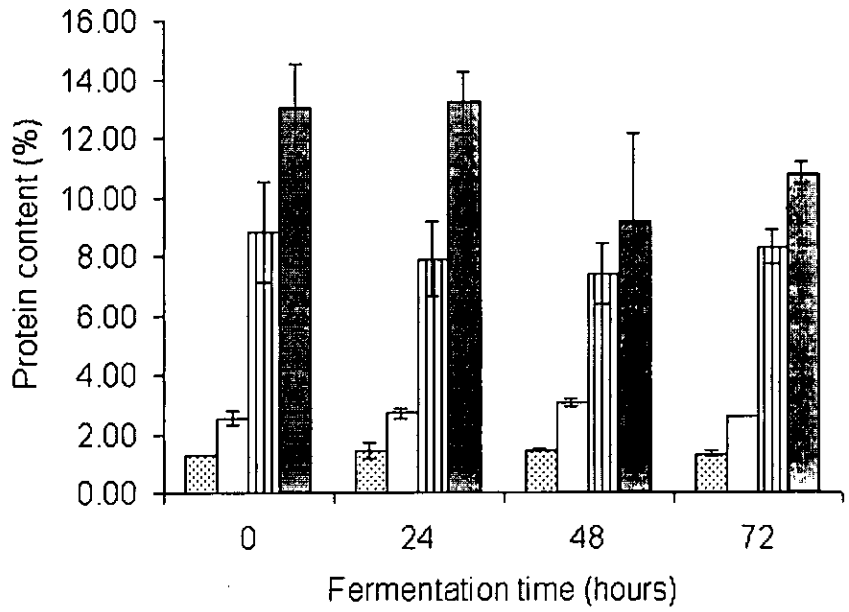

Figure 4.11 Changes in protein content during fermentation of cassava roots uninoculated. Cassava roots only (▨); Cassava + cowpea, 96:4 (□); Cassava + cowpea, 92:8 (▥); Cassava + cowpea, 88:12 (□). Error bars represent the standard deviation of 2 independent samples

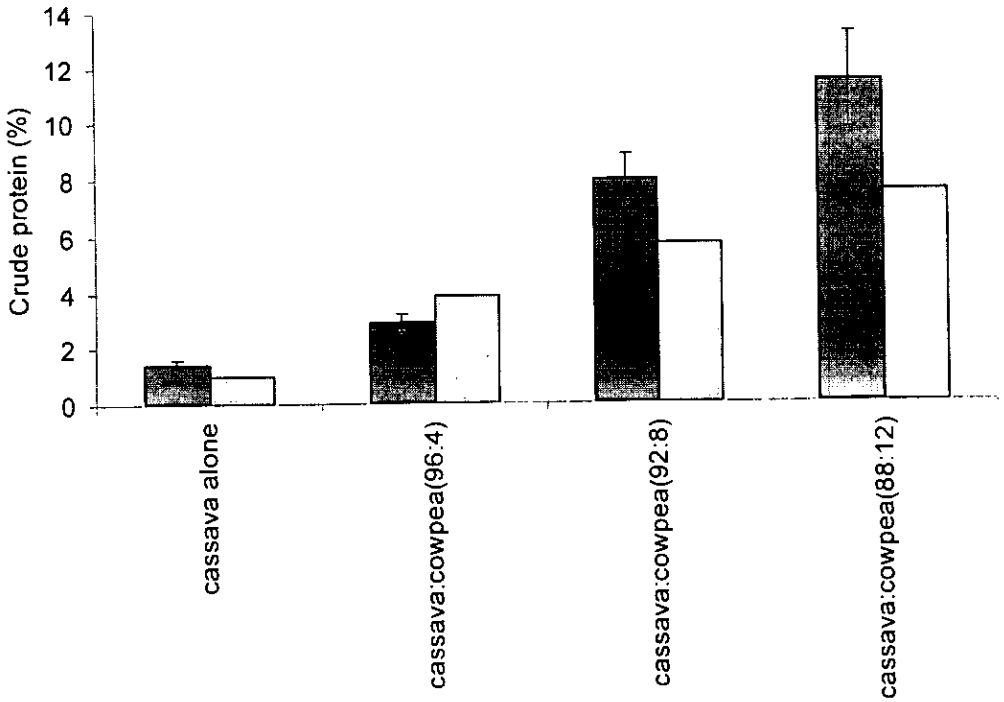

Figure 4.12 Protein contents of cassava flour supplemented with different quantities of cowpea flour. Experimental value (■) and Predicted value (□). Error bars represent the standard deviation of 2 independent samples

4.4.4 Ash content

The ash content results are expressed on a dry weight basis. For flour from unfermented cassava roots (fermentation time 0 hours), there was no significant increase of ash content ($p > 0.05$) with increase in cowpea flour added to the cassava roots (Table 4.10). For flour from unfermented cassava roots without cowpea supplementation, the average ash content was $3.0 \pm 0.1\%$ and for flour from unfermented cassava roots with cowpea supplementation, the average ash content was $3.1 \pm 0.4\%$.

For cassava flour from uninoculated samples, either supplemented with cowpea or not, there was no significant difference in ash content ($p > 0.05$) between flour from unfermented samples and that from samples incubated at 30 °C up to 72 hours.

For cassava flour from inoculated cassava roots, there was a significant increase of ash content ($p < 0.05$) with fermentation time (Table 4.10). For cassava flour from unfermented cassava roots the average ash content was 3.2 ± 0 % and for fermented samples up to 72 hours, the average ash content was 3.7 ± 0.1%.

Table 4.10 Total ash content (%) of unfermented and fermented cassava flour prepared from cassava and cassava + cowpea

Treatment	Fermentation time (hours)	
	0	72
Cassava, uninoculated	2.9	2.70 ± 0.1
Cassava + cowpea (96:4), uninoculated	2.9 ± 0.1	2.69
Cassava + cowpea (92:8), uninoculated	2.7	2.70
Cassava + cowpea (88:12), uninoculated	2.6 ± 0.1	2.89 ± 0.1
Cassava alone + *R. stolonifer*	3.0 ± 0.1	3.84 ± 0.1
Cassava + cowpea (96:4) + *R. stolonifer*	3.1	3.83 ± 0.1
Cassava + cowpea (92:8) + *R. stolonifer*	3.2	3.59
Cassava + cowpea (88:12) + *R. stolonifer*	3.2 ± 0.1	3.66 ± 0.1

Average and standard deviation from 2 independent cassava flour samples

4.5 EFFECT OF CO-FERMENTATION OF CASSAVA WITH COWPEA ON THE TEXTURE OF CASSAVA ROOTS AND VISCOSITY OF CASSAVA FLOUR PASTE

4.5.1 Growth of *Rhizopus stolonifer* (*R. stolonifer*) and *Neurospora sitophila* (*N. sitophila*)

Cassava roots inoculated with *Rhizopus stolonifer* were moderately covered with white mycelia after 24 hours of fermentation and were fully covered with mycelia and black spores after 72 hours of fermentation (Table 4.11). In cassava roots supplemented with cowpea, the growth of *Rhizopus stolonifer* mycelia was faster than in cassava roots without cowpea supplementation at all the different fermentations periods. *Rhizopus stolonifer* mycelia covered the roots supplemented with cowpea after 48 hours.

Cassava roots inoculated with *Neurospora sitophila*, without cowpea supplementation, were almost covered with white mycelia after 24 hours of fermentation. In the same roots, *N. sitophila* mycelia, with orange spores, totally covered the roots after 48 hours of fermentation. In cassava roots supplemented with cowpea, the *Neurospora sitophila* mycelia fully covered the roots with high production of orange spores after 24 hours of fermentation. Growth of *Neurospora sitophila* was faster than that of *Rhizopus stolonifer* in both the cowpea supplemented and unsupplemented cassava roots (Table 4.11). The fermentations in this experiment were characterized by production of unpleasant smells in cassava roots supplemented with cowpea and inoculated with either mould. No fungal growth was observed in uninoculated cassava roots, either supplemented with cowpea or not.

TABLE 4.11 Growth of *Rhizopus stolonifer* (*R. stolonifer*) (CNF6) and *Neurospora sitophila* (*N. sitophila*) (CNF7) during fermentation of cassava roots, either supplemented with cowpea or not at 30° C

	Fermentation time (hours)			
Sample	**0**	**24**	**48**	**72**
Cassava, uninoculated	0	0	0	0
Cassava + *R. stolonifer*	0	2	3	4
Cassava + *N. sitophila*	0	3	4	4
Cassava + cowpea, uninoculated	0	0	0	0
Cassava + cowpea+ *R. stolonifer*	0	3	4	4
Cassava+cowpea+*N. Sitophila*	0	4	4	4

0- No growth (no mycelia), **1**- Slight growth (less than 1/4 of the roots covered with mycelia), **2**- Moderate growth (more than 1/4 and less than 1/2 of the roots covered with mycelia), **3**- Growth (more than 1/2 of the roots covered with mycelia), **4**- Good growth (all roots covered with mycelia)

4.5.2 Change in softness of cassava roots during fermentation

In uninoculated cassava roots without cowpea supplementation, there was no significant change in hardness throughout the fermentation period (Figure 4.13). In uninoculated cassava roots supplemented with cowpea, decrease in hardness was observed after 24 hours of incubation from 2000 $\pm$ 200 to 1780 $\pm$ 50 g peak force, then remained constant for the rest of fermentation periods. Uninoculated cassava roots supplemented with cowpea were significantly softer than uninoculated cassava roots without cowpea supplementation ($P < 0.001$) for samples fermented up to 24 and up to 72 hours.

Fermentation with *Rhizopus stolonifer* and *Neurospora sitophila* increased the softness of cassava roots, either supplemented with cowpea or not. In the inoculated cassava roots, the highest increase in softness was observed between 24 and 48 hours of fermentation. During this period, the hardness of cassava roots unsupplemented with cowpea decreased from 1430 $\pm$ 60 and 1900 $\pm$ 100 to 760 $\pm$ 60 and 830 $\pm$ 100 g of peak force, when cassava roots were inoculated

with *Rhizopus stolonifer* and *Neurospora sitophila* respectively. In the same period, the hardness of cassava roots supplemented with cowpea decreased from 1270 ± 90 and 1560 ± 60 to 130 ± 9 and 820 ± 100 g of peak force, when cassava roots were inoculated with *Rhizopus stolonifer* and *Neurospora sitophila* respectively.

For samples fermented up to 48 hours, cassava roots supplemented with cowpea and inoculated with *Rhizopus stolonifer* were significantly softer than all other inoculated samples ($p < 0.001$). In cassava roots inoculated with *Neurospora sitophila*, there was no significant difference in hardness ($p > 0.05$) between cassava roots that were supplemented with cowpea and cassava roots that were not supplemented.

After 72 hours of fermentation, there was no significant difference in softness ($p > 0.05$) between cowpea supplemented cassava roots that were inoculated with *Rhizopus stolonifer* and cassava roots inoculated *Rhizopus stolonifer* without cowpea supplementetion. However, in cassava roots inoculated with *Neurospora sitophila*, the samples supplemented with cowpea were significantly softer than the samples unsupplemented with cowpea ($p < 0.05$).

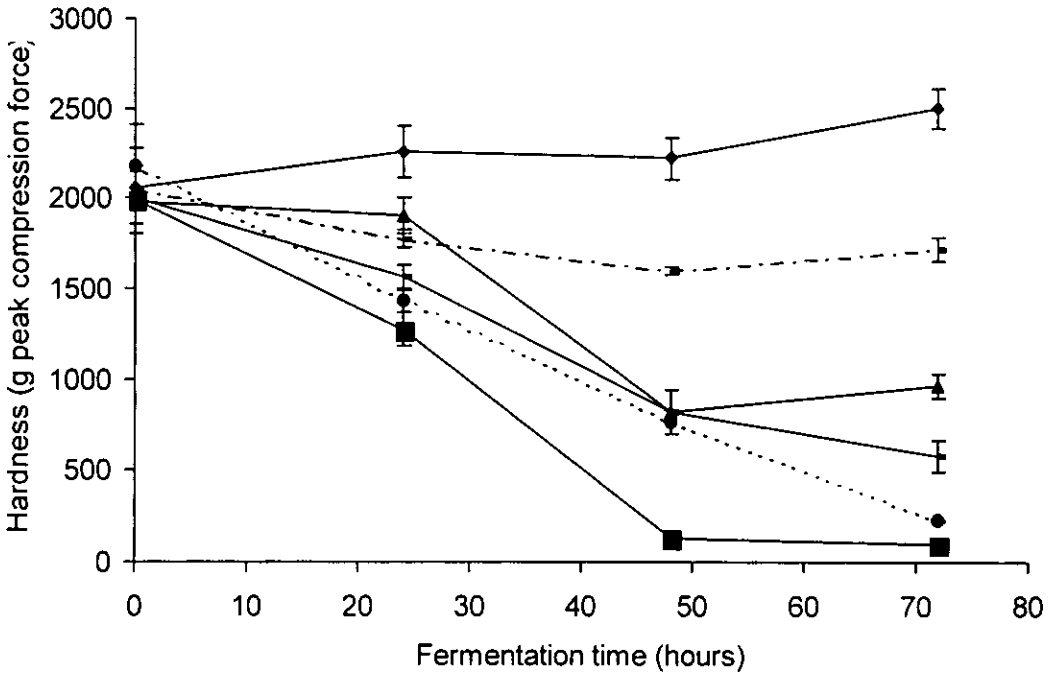

Figure 4.13 Hardness measured with Stable Micro Systems Texture Analyser (SMS - England) of cassava roots during fermentation with *Rhizopus stolonifer* and *Neurospora sitophila* at 30 °C. Cassava, uninoculated (—◆—),Cassava, inoculated with *Rhizopus stolonifer* (···●···),Cassava, inoculated with *Neurospora sitophila* (—▲—),Cassava + cowpea, uninoculated (–·–·–), Cassava + cowpea, inoculated with *Rhizopus stolonifer* (—■—) Cassava + cowpea, inoculated with *Neurospora sitophila* (——).Error bars represent the standard deviation of 2 independent samples

4.5.3 Effect of mould fermentation and cowpea supplementation of cassava roots on the viscosity of cassava flour paste

Viscosity of paste from fermented cassava roots, unsupplemented with cowpea

For paste prepared from uninoculated cassava flour, there was no significant change in viscosity throughout all the fermentation periods. For paste from mould inoculated cassava

roots, there were no significant differences in viscosity between samples inoculated with either *Rhizopus stolonifer* or *Neurospora sitophila* ($p > 0.05$) (Figure 4.14). Fermentation of cassava roots inoculated with moulds up to 24 hours did not significantly affect the viscosity of cassava flour paste. The viscosity of cassava flour from cassava roots fermented with *Rhizopus stolonifer* up to 72 hours was significantly lower ($p < 0.05$) than that from cassava roots fermented up to 24 hours. There was no significant change in the viscosity of paste from fermented cassava roots inoculated with *Neurospora sitophila* throughout all the fermentation periods ($p > 0.05$).

Viscosity of paste from fermented cassava roots supplemented with cowpea

Cassava roots supplemented with cowpea produced cassava flour pastes with extremely low viscosity, with an average Hagberg falling number of 89 $\pm$ 20 s compared to 280 $\pm$ 20 s in paste from cassava flour without cowpea supplementation.

Fermentation of cassava roots supplemented with cowpea up to 24 hours resulted in a significant increase of viscosity of the paste for all cassava flour samples from an average Hagberg falling number of 70 $\pm$ 2 to 119 $\pm$ 10 s ($p< 0.001$). Samples inoculated with *Rhizopus stolonifer* and *Neurospora sitophila* and fermented up to 72 hours, produced flour pastes with significantly lower viscosity (falling number range 68 $\pm$ 1 and 75 $\pm$ 1 s) than cassava flour paste from cassava roots fermented up to 24 hours (falling number range 137 $\pm$ 2 and 113 $\pm$ 1 s) (Figure 4.14). Uninoculated cassava roots supplemented with cowpea resulted in no significant change in viscosity of paste from cassava roots fermented up to 24 and up to 72 hours. There was no significant difference in viscosity between pastes of cassava flour from unfermented cassava roots supplemented with cowpea with that from cassava roots fermented up to 72 hours.

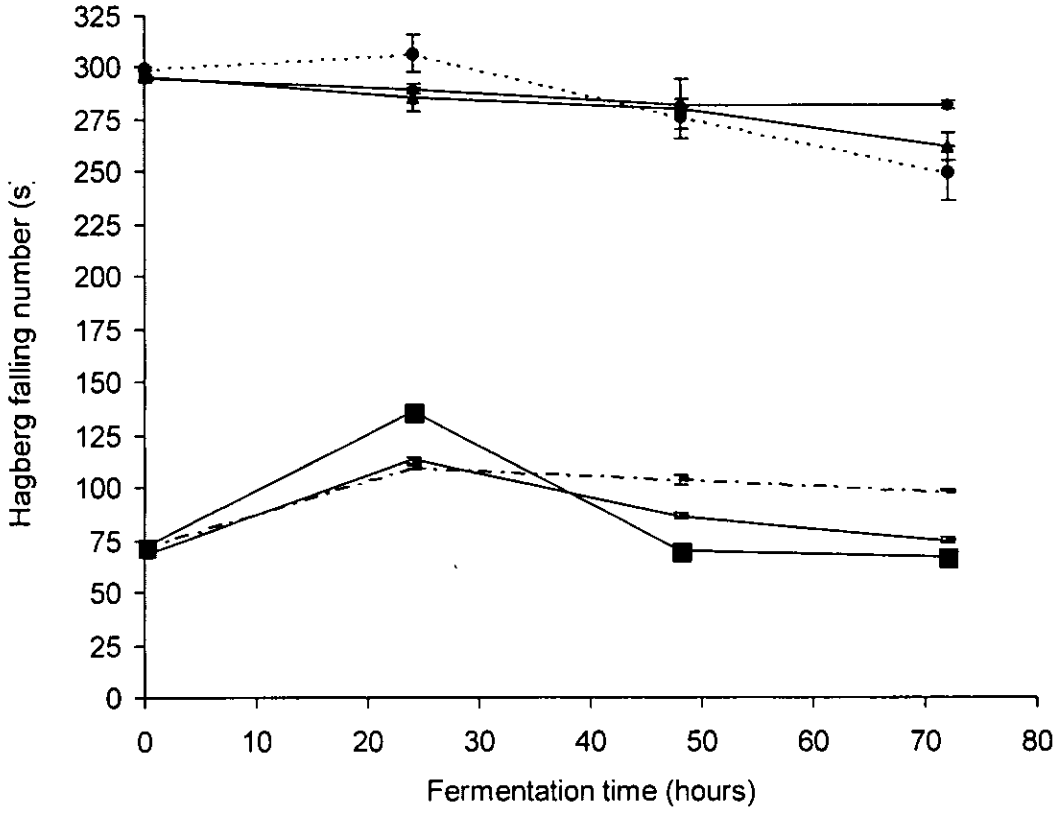

Figure 4.14 Change in viscosity (Hagberg falling number) of cassava during fermentation with *Rhizopus stolonifer* and *Neurospora sitophila*. Cassava, uninoculated (—◆—), Cassava, inoculated with *Rhizopus stolonifer* (···●···),Cassava, inoculated with *Neurospora sitophila* (—▲—),Cassava + cowpea, uninoculated (– · – · –), Cassava + cowpea, inoculated with *Rhizopus stolonifer* (—■—) Cassava + cowpea, inoculated with *Neurospora sitophila* (——). Error bars represent the standard deviation of 2 independent samples

4.6 SENSORY EVALUATION OF PASTE PREPARED USING FLOUR FROM CASSAVA ROOTS CO-FERMENTED WITH COWPEA

4.6.1 Evaluation of the acceptability of the appearance of *karakata* prepared from different cassava flour samples

There was no significant difference in the acceptability of the appearance ($p > 0.05$) of *karakata* prepared from fermented cassava flour samples that were not supplemented with cowpea. Scores of *karakata* prepared from laboratory fermented cassava flour without cowpea supplementation were in the range 5.3 ± 2 to 5.5 ± 2 while those for *karakata* prepared from the 2 locally fermented samples ranged from 4.3 ± 2 to 5.2 ± 2 (Figure 4.15).

There was a significant difference in acceptability of the appearance ($p < 0.01$) of *karakata* prepared from fermented cassava flour without cowpea and unfermented cassava flour, either supplemented with cowpea or not (score range 7.6 ± 1 to 8.5 ± 1). Panellists preferred *karakata* from unfermented flour to that from fermented flour. However, there was no significant difference in acceptability of appearance ($p > 0.05$) between *karakata* prepared from fermented cassava flour supplemented with cowpea (score range 6.6 ± 1.6 to 7.7 ± 0.9) and that prepared from unfermented flour.

The appearance of *karakata* from unfermented cassava flours and fermented cassava flours supplemented with cowpea (score range 6.6 ± 1.6 to 8.5 ± 0.5) was significantly more acceptable ($p < 0.05$) compared to that prepared from fermented cassava flours that were not supplemented with cowpea (score range 4.3 ± 1.7 to 5.5 ± 1.6) (Figure 4.15).

There was no significant difference in the appearance ($p > 0.05$) of *karakata* prepared from flour fermented with either *Rhizopus stolonifer*, *Neurospora sitophila* or with both fungi.

The *karakata* prepared from the local fermented cassava flours was a very dark grey and that from laboratory fermented cassava flours without cowpea supplementation, inoculated with

Rhizopus stolonifer, *Neurospora sitophila* and both *Rhizopus stolonifer* and *Neurospora sitophila* in the laboratory was light brown, in spite of the *Rhizopus stolonifer* producing the black spores.

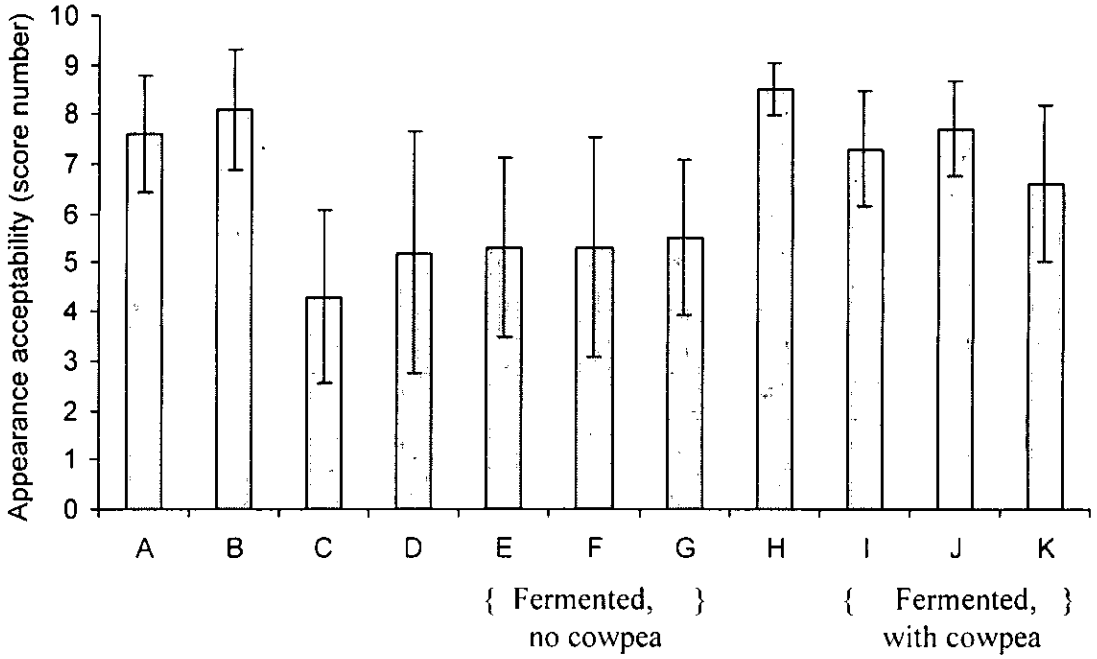

Figure 4.15 General appearance acceptability scores (1- dislike very much to 9- like very much) of *karakata* prepared from different cassava flours. Error bars represent the standard deviation of the scores of 10 panellists

Key
Cassava flour reference:

A- Unfermented from local market, **B**- Unfermented from laboratory, **C**- Fermented, from local market 1, **D**- Fermented, from local market 2, **E**- Fermented with *R. stolonifer*, **F**- Fermented with *N. sitophila*, **G**- Fermented with a mixture of *R. stolonifer* and *N. sitophila*, **H**- Unfermented, supplemented with cowpea, **I**- Supplemented with cowpea and fermented with *R. stolonifer*, **J**- Supplemented with cowpea and fermented with *N. sitophila*, **K**- Supplemented with cowpea and fermented with a mixture of *R. stolonifer* and *N. sitophila*.

4.6.2 Evaluation of the acceptability of the odour of *karakata* prepared from different cassava flour samples

For *karakata* prepared from the unfermented cassava flours, either supplemented with cowpea or not, there was no significant difference in acceptability of odour ($p > 0.05$) (score range 7.4 ± 2 to 8.2 ± 1) (Figure 4.16). For the cassava flours without cowpea supplementation, *karakata* prepared from unfermented flour samples was significantly more acceptable ($p < 0.01$) than that prepared from flours from fermented cassava roots using either *Rhizopus stolonifer* or *Neurospora sitophila* alone or a combination of the moulds (score range 4.4 ± 2 to 5.2 ± 2). There was no significant difference in odour acceptability ($p > 0.05$) of *karakata* from the unfermented cassava flours and that from fermented cassava flours supplemented with cowpea (score range 6.1 ± 2 to 7.4 ± 1). *Karakata* from fermented cassava flours that were supplemented with cowpea and inoculated with both moulds was significantly more acceptable ($p < 0.05$) than that from the two local fermented flours and that from the fermented cassava flours from roots inoculated with each single mould (Figure 4.16).

For flour samples without cowpea supplementation, there was no significant difference in odour acceptability ($p > 0.05$) of *karakata* prepared from flour from cassava roots fermented with either *Rhizopus stolonifer, Neurospora sitophila* or with both moulds. For flour samples with cowpea supplementation, there was no significant difference in odour acceptability ($p > 0.05$) of *karakata* prepared from flour from fermented cassava roots inoculated with *Rhizopus stolonifer*, inoculated with *Neurospora sitophila* and inoculated with a combination of both moulds.

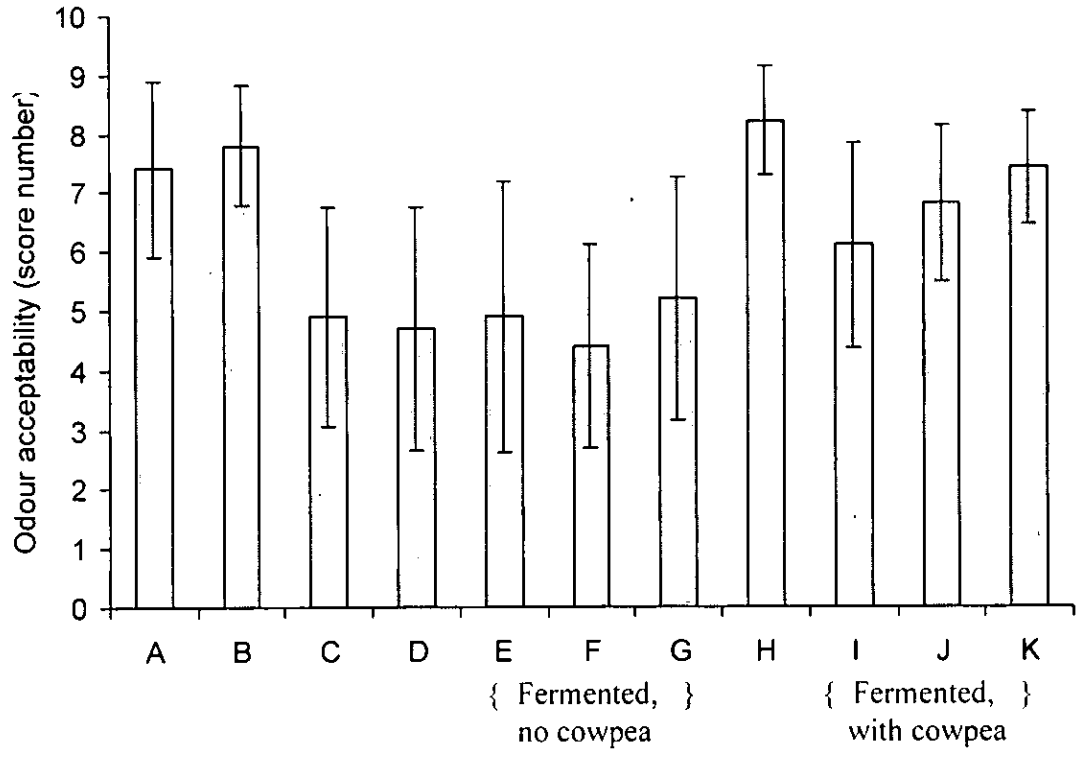

Figure 4.16 General odour acceptability scores (1- Very bad to 9-Very good) of *karakata* prepared from different cassava flours. Error bars represent the standard deviation of the scores of 10 panellists

Key
Cassava flour reference:

A- Unfermented from local market, **B**- Unfermented from laboratory, **C**- Fermented, from local market 1, **D**- Fermented, from local market 2, **E**- Fermented with *R. stolonifer*, **F**- Fermented with *N. sitophila*, **G**- Fermented with a mixture of *R. stolonifer* and *N. sitophila*, **H**- Unfermented, supplemented with cowpea, **I**- Supplemented with cowpea and fermented with *R. stolonifer*, **J**- Supplemented with cowpea and fermented with *N. sitophila*, **K**- Supplemented with cowpea and fermented with a mixture of *R. stolonifer* and *N. sitophila*.

4.6.3 Evaluation of the acceptability of the taste of *karakata* prepared from different cassava flour samples

For samples without cowpea supplementation, there was no significant difference in taste acceptability ($p > 0.05$) between *karakata* from laboratory fermented flour and that from locally bought fermented cassava flours (score range 3.9 ± 2 to 5.0 ± 2) (Figure 4.17). *Karakata* prepared from unfermented flours, either supplemented with cowpea or not (score range 7.3 ± 1 to 8.3 ± 0) was significantly more acceptable in taste ($p < 0.01$) than that from fermented cassava flours without cowpea supplementation (Figure 4.17). There was no significant difference in taste acceptability ($p > 0.05$) between *karakata* prepared from unfermented cassava flours and that prepared from fermented cassava flours supplemented with cowpea (score range 6.3 ± 1.3 to 8.1 ± 0). *Karakata* prepared from fermented cassava flour supplemented with cowpea, except that inoculated with both moulds, was significantly more acceptable in taste ($p < 0.01$) than that from fermented cassava without cowpea supplementation (Figure 4.17). *Karakata* from laboratory prepared cassava flour with cowpea supplementation was more acceptable than that from the locally bought fermented flours.

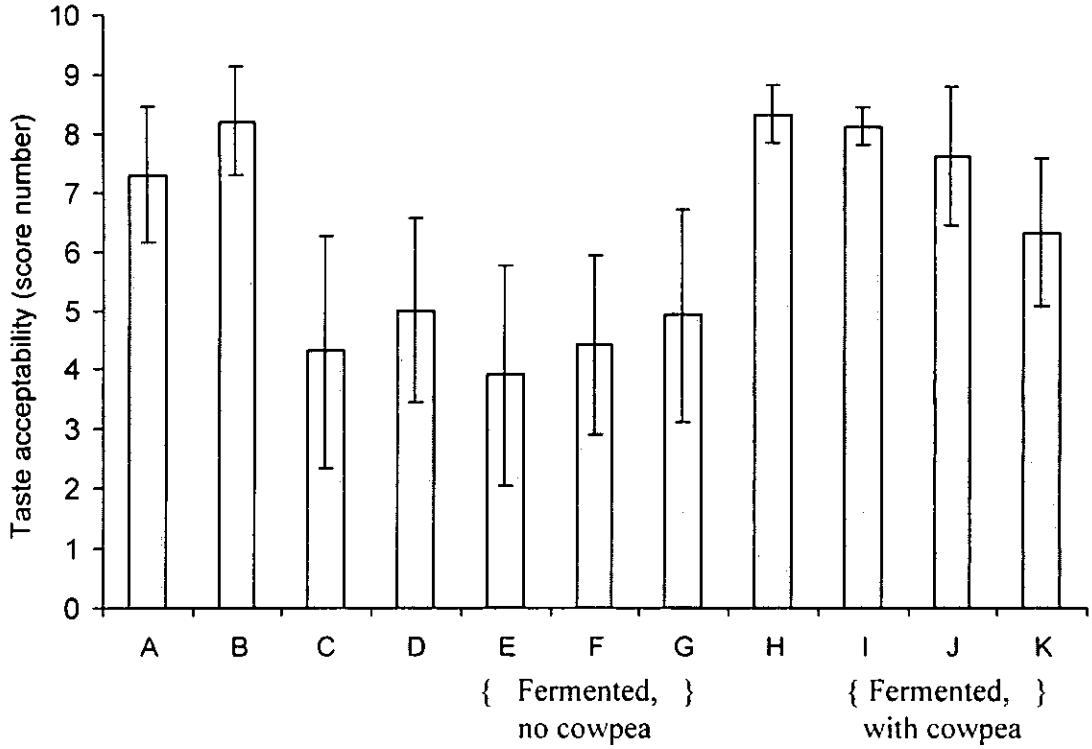

Figure 4.17 General taste acceptability scores (1- dislike very much to 9- like very much) of *karakata* prepared from different cassava flours. Error bars represent the standard deviation of the scores of 10 panellists

Key
Cassava flour reference:

A- Unfermented from local market, **B-** Unfermented from laboratory, **C-** Fermented, from local market 1, **D-** Fermented, from local market 2, **E-** Fermented with *R. stolonifer*, **F-** Fermented with *N. sitophila*, **G-** Fermented with a mixture of *R. stolonifer* and *N. sitophila*, **H-** Unfermented, supplemented with cowpea, **I-** Supplemented with cowpea and fermented with *R. stolonifer*, **J-** Supplemented with cowpea and fermented with *N. sitophila*, **K-** Supplemented with cowpea and fermented with a mixture of *R. stolonifer* and *N. sitophila*

In a test for flavour, *karakata* from fermented cassava flours inoculated with isolated moulds and unsupplemented with cowpea, was considered bitter by more than 60% of the panellists (Table 4.12). *Karakata* from unfermented cassava flour was considered sweet by 60 to 70% of the panellists and that from fermented cassava flour supplemented with cowpea was considered

sweet by 60% of the panellists when inoculated with *R. stolonifer*. When inoculated with *N. sitophila* or both *Rhizopus stolonifer* and *Neurospora sitophila* as starters, *karakata* was considered as neither bitter nor sweet by 60% of the panellists (Table 4.12).

Table 4.12 Identification of flavour of *karakata* by the panellist

	Number of responses for each sample from the 10 panellists										
	Unfer. Local market	**Unfer. Laboratory**	**Fermented, local market**		**Fermented, without cowpea**			**Unfer. with cowpea**	**Fermented, with cowpea**		
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**
Flavor											
Sweet	6	7	3	3	2	2	2	5	6	4	2
Bitter	2	1	0	0	6	7	7	0	0	0	0
Sour	2	0	3	2	0	0	1	0	0	0	2
Neither sweet nor bitter	0	2	4	5	2	1	0	5	4	6	6
I don't Know	0	0	0	0	0	0	0	0	0	0	0

Unfer. Unfermented
Fer. Fermented

Key
Cassava flour reference:

A- Unfermented from local market, **B**- Unfermented from laboratory, **C**- Fermented, from local market 1, **D**- Fermented, from local market 2, **E**- Fermented with *R. stolonifer*, **F**- Fermented with *N. sitophila*, **G**- Fermented with a mixture of *R. stolonifer* and *N. sitophila*, **H**- Unfermented, supplemented with cowpea, **I**- Supplemented with cowpea and fermented with *R. stolonifer*, **J**- Supplemented with cowpea and fermented with *N. sitophila*, **K**- Supplemented with cowpea and fermented with a mixture of *R. stolonifer* and *N. sitophila*

4.6.4 Evaluation of the acceptability of the texture of *karakata* prepared from different cassava flour samples

Texture acceptability was evaluated according to the feel of the thick porridge on touching and chewing. There was no significant difference in texture acceptability ($p > 0.05$) between *karakata* from unfermented cassava flour (score range 7.4 ± 1.8 to 8.4 ± 1.0), and that from flour from fermented cassava roots supplemented with cowpea and inoculated *Rhizopus stolonifer* and *Neurospora sitophila* separately (score range 7.5 ± 1.0 to 7.7 ± 0.9) (Figure 4.18). The texture of *karakata* from unfermented cassava flours from the laboratory, either supplemented with cowpea or not, (score range 8.3 ± 0.5 to 8.4 ± 1) was significantly more acceptable ($p < 0.05$) than that from fermented cassava flour supplemented with cowpea and inoculated with a combination of both moulds (score 6.0 ± 1.8). The was no significant difference in texture acceptability ($p > 0.05$) between *karakata* prepared from laboratory fermented cassava flour without cowpea supplementation and the two locally fermented cassava flours (score range 4.1 ± 1.4 to 5.6 ± 1.4). The texture of *karakata* prepared from the fermented cassava flour without cowpea supplementation, except that prepared from local flour 2, was significantly less acceptable ($p < 0.01$) than that prepared from unfermented cassava flour, either supplemented with cowpea or not, and that prepared from fermented cassava flour supplemented with cowpea. There was no significant difference in texture acceptability ($p > 0.05$) between *karakata* prepared from local fermented cassava flour 2 (score 5.6 ± 1.4) and that from flour supplemented with cowpea and fermented with a combination of *Rhizopus stolonifer* and *Neurospora sitophila* (score 6.0 ± 1.8).

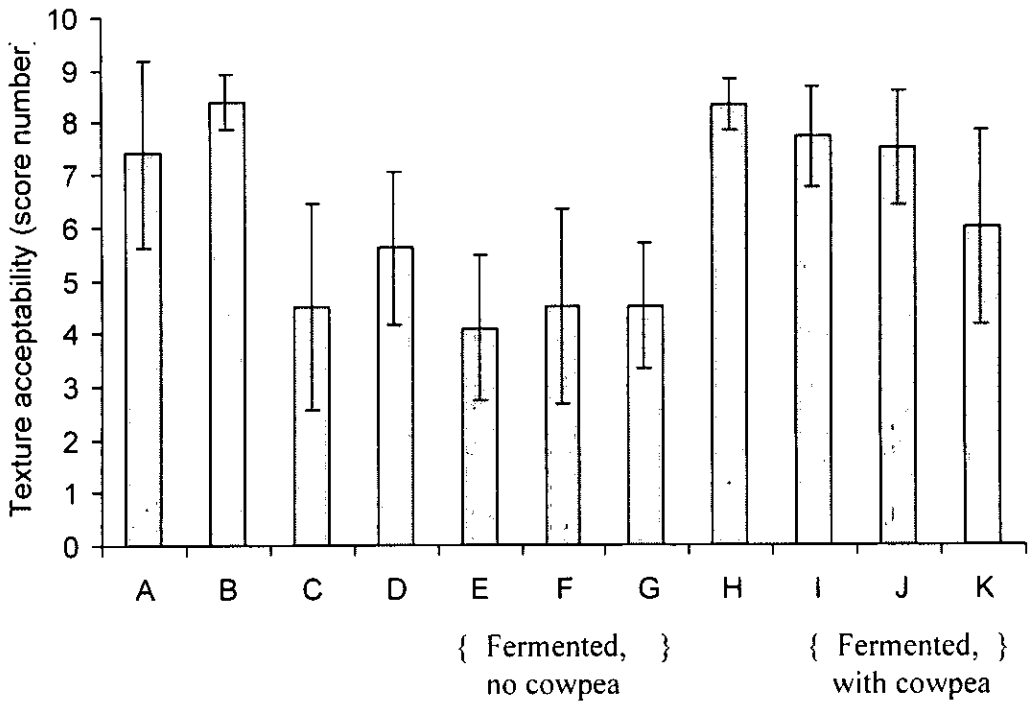

Figure 4.18 General texture acceptability scores (1- very bad to 9- very good) of *karakata* prepared from different cassava flours. Error bars represent the standard deviation of the scores of 10 panellists

Key
Cassava flour reference:

A- Unfermented from local market, **B**- Unfermented from laboratory, **C**- Fermented, from local market 1, **D**- Fermented, from local market 2, **E**- Fermented with *R. stolonifer*, **F**- Fermented with *N. sitophila*, **G**- Fermented with a mixture of *R. stolonifer* and *N. sitophila*, **H**- Unfermented, supplemented with cowpea, **I**- Supplemented with cowpea and fermented with *R. stolonifer*, **J**- Supplemented with cowpea and fermented with *N. sitophila*, **K**- Supplemented with cowpea and fermented with a mixture of *R. stolonifer* and *N. sitophila*.

4.6.5 Cyanogenic potential

Sun drying of unfermented fresh cassava roots reduced the cyanogenic potential by 50%. The initial cyanogenic potential in fresh cassava roots was 62 ± 10 mg HCN/kg (Figure 4.19). Sun drying of samples of unfermented cassava roots supplemented with cowpea resulted in cyanogenic potential of 24.6 mg HCN/kg.

Where cassava roots unsupplemented with cowpea were fermented and sun dried, cyanogenic potential in flour was reduced by 80% when inoculated with *Rhizopus stolonifer*, by 87% when inoculated with *Neurospora sitophila*, and by 87% when inoculated with both moulds.

For cassava roots supplemented with cowpea, the cyanogenic potential in flours were 5.3 ± 1 mg HCN/kg, for cassava roots fermented with *Rhizopus stolonifer*, 6.7 mg HCN/kg for cassava roots fermented with *Neurospora sitophila* and 4 ± 2.8 mg HCN/kg for cassava roots fermented with both moulds.

In cassava flours collected in Nampula city to be used in sensory evaluation of *karakata*, the cyanogenic potential were 21 ± 1, 26 ± 3 and 15 ± 1 mg HCN/kg for unfermented cassava flour, fermented cassava flour 1 and fermented cassava flour 2 respectively.

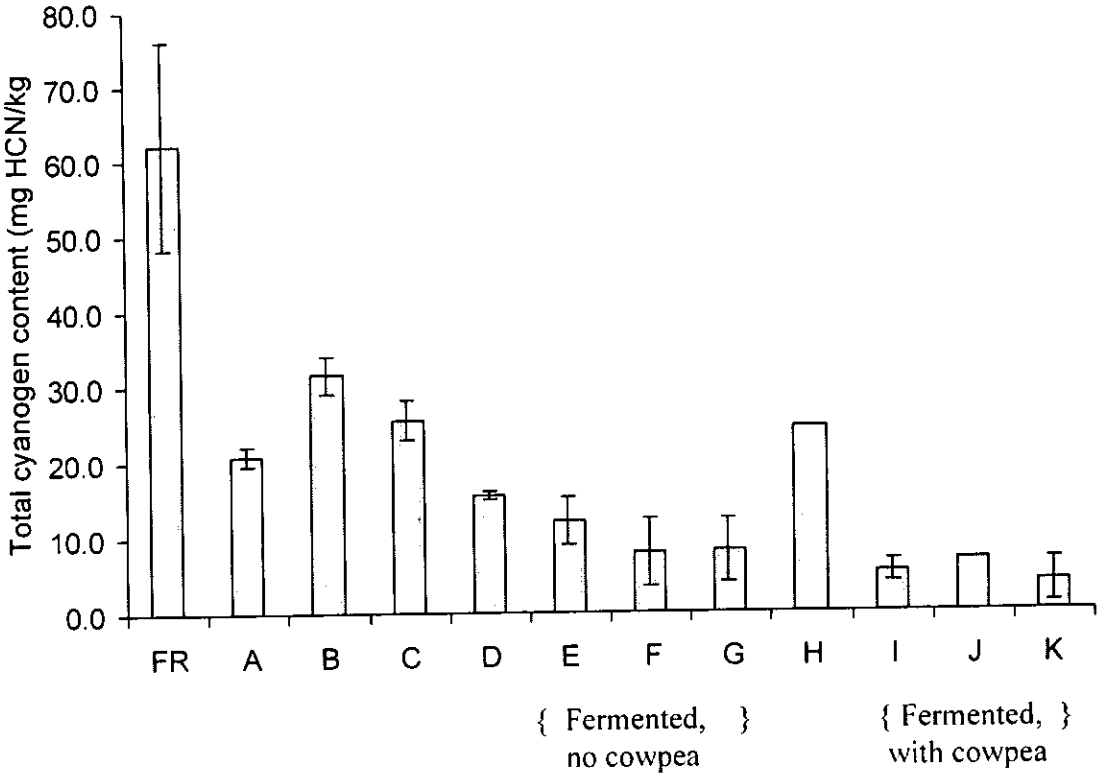

Figure 4.19 Cyanogenic potential of different cassava flours. Error bars represent the standard deviation of 2 independent determinations

Key

FR – Fresh cassava roots used to prepare cassava flour in the laboratory.

Cassava flour reference:

A- Unfermented from local market, **B**- Unfermented from laboratory, **C**- Fermented, from local market 1, **D**- Fermented, from local market 2, **E**- Fermented with *R. stolonifer*, **F**- Fermented with *N. sitophila*, **G**- Fermented with a mixture of *R. stolonifer* and *N. sitophila*, **H**- Unfermented, supplemented with cowpea, **I**- Supplemented with cowpea and fermented with *R. stolonifer*, **J**- Supplemented with cowpea and fermented with *N. sitophila*, **K**- Supplemented with cowpea and fermented with a mixture of *R. stolonifer* and *N. sitophila*

5.0 DISCUSSION

5.1 CYANOGENIC POTENTIAL OF CASSAVA FLOUR COLLECTED FROM SELECTED DISTRICTS OF NAMPULA PROVINCE, MOZAMBIQUE

Cassava processing by heap fermentation followed by sun drying gave substantially lower cyanogen contents compared with sun drying only. The results are similar to those obtained by other workers (Bradbury, 1997; Cardoso *et al.*, 1998 and Ernesto *et al.*, 2000) for Memba district and Mogincual districts. Cardoso *et al.*, 1998 and Ernesto *et al.*, 2000 reported that the total cyanogens levels of flour produced by heap fermentation were only about 50% of those obtained after processing by sun drying only.

The distribution curve of the cyanogenic potentials of all the samples of cassava flour showed downward curvature, indicating that most of the samples had low cyanogenic potential values (0 - 20 HCN mg/Kg) while few samples had high values (> 100 mg HCN/kg). The distribution did not show multiple peaks in contrast to the results obtained by Cardoso *et al.* (1998) in Mogincual district. The difference may be because the flour samples in this study were collected in July while the samples reported by Cardoso *et al.* (1998) were collected in October, which is the harvest period. Most of the cassava flour samples collected in July were processed from cassava roots harvested during the previous harvest season while the cassava flour collected in October were processed from cassava roots that were harvested in that current season. Storage of cassava chips has been reported to lead to a decrease in residual cyanogenic glucosides of processed cassava flour with time (Essers and Nout, 1989).

In Mogincual district, the average cyanogenic potential value of cassava flour processed from unfermented cassava roots was lower than that of cassava flour processed from fermented

cassava roots because four out of the seven samples from unfermented roots were prepared from sweet varieties of cassava roots which have been reported to generally have low cyanogenic potentials (Cardoso *et al.*, 1998). During the civil war in 1992, Mogincual district was affected by epidemic spastic paraparesis, *konzo*, associated with consumption of bitter cassava roots that were inadequately processed (Cliff, 1994). It has been observed that the production and processing of sweet cassava varieties has increased in the district since the end of the war as the population is aware of the consequences of consuming inadequately processed bitter varieties (Ernesto *et al.*, 2000).

From the twenty seven samples of cassava flour collected in Mogincual district, only one sample prepared from the sweet variety *Uhope*, had a cyanogenic potential value below 10 mg HCN/kg, the value regarded by the Code Alimentarius Commission of the FAO/WHO (1991) as safe. According to work carried out by Cardoso *et al.* (2005), to produce cassava flour of 10 mg HCN/kg by sun drying or heap fermentation requires starting with sweet varieties containing 12 – 32 mg HCN/kg of cyanogenic potential. Heap fermentation and sun drying do not adequately remove cyanide in the bitter cassava varieties. New and greatly improved processing methods are thus required in order to produce safe cassava products. It would also be important to attempt to introduce low cyanogenic potential, high yielding and ecologically adapted cassava varieties.

The average cyanogenic potential values for the four districts were all above 10 mg HCN/kg. Most of the cassava flour samples collected were from bitter cassava varieties. The existence of cassava flour sample with high cyanogenic potential values in the four districts may justify the recent sporadic cases of *konzo* reported in Nampula Province (Ernesto *et al.*, 2002).

The high cyanogenic potential values obtained in Memba district, similar to those obtained by Ernesto *et al.* (2000), 96 $\pm$ 45 mg HCN/kg may have been a result of shortcuts in

the processing regime. Shortcuts in the processing are usually common in times when food supply is low. During the time of collection of samples for this study, the production of cassava roots was low due to viral infection of the cassava roots. The short food supply in Memba district may also be because the district is located in an area which is difficult to access and has thus received little assistance from agricultural and health government and non governmental agents. The highest number of *konzo* cases reported in Mozambique in 1981-1982, 1102 cases, occurred in Memba district. The disease was associated with consumption of insufficiently processed bitter cassava varieties during drought and war (Casadei *et al.*, 1990; Cliff, 1994).

5.2 MICROBIAL AND BIOCHEMICAL CHARACTERISTICS OF CASSAVA HEAP FERMENTATION PROCESS

The method of heap fermentation observed in Nampula Province, Mozambique, differed from the one used in Uganda, described by Essers *et al.* (1995a), in that, in Nampula, sun-drying was not done before heaping and covering. High ambient temperatures of 30 to 34 °C, force the local peasants to carry out heap fermentation in a place where there is shade, which avoids rapid decrease of moisture on cassava roots. Covering the roots with cassava peels and leaves also prevented rapid loss of moisture in cassava roots. In Nampula, heap fermentation was usually carried out during the dry season. During midday, the temperature inside the heaps was lower than the temperature outside during the fermentation. Evaporation of the moisture, as observed by condensation of water on the surface of leaves and cassava peels used to cover cassava roots, lowered the temperature inside the heap.

The decrease in the pH of cassava as fermentation progressed was probably caused by production of lactic acid by bacteria. The pH was in the range between 5 and 6, which is ideal for cyanogenic glucoside break down (White *et al.*, 1994).

The fermentation of cassava roots was dominated by *Rhizopus stolonifer*, the black mould, which grows well in water activity greater than 0.93 and temperatures between 25 to 30 °C (Pitt and Hocking, 1997).

The genus of lactic acid bacteria (LAB), *Leuconostoc*, which was isolated in this study, has also been isolated in cassava fermentation for *gari*, *fufu* and *akyeke* preparation in West Africa (Westby and Twiddy, 1992; Obilie *et al.*, 2004). The LAB play a role in determining the overall flavour of fermented cassava products. *Leuconostoc mesenteroides* is responsible for the souring of cassava products such as *akyeke* and *gari* (Westby and Twiddy, 1992; Obilie *et al.*, 2004).

The moulds, *Rhizopus stolonifer* and *Neurosopora sitophila*, isolated in this study, were isolated and identified by Essers *et al.* (1995a) from heap fermented cassava roots. In the present study it was observed that the fermentation of cassava roots was dominated by *R. stolonifer*. Moulds play an important role in reducing the total cyanogens in cassava chips (Essers *et al.*, 1995b). The growth of moulds degrades the cell wall of cassava roots, enabling contact between linamarin and linamarase. Linamarin is hydrolyzed to form cyanohydrins and subsequently to volatile HCN at ambient temperature (Essers *et al.*, 1995b).

Although fermentation plays an important role in reducing the total cyanogens in cassava chips, the cyanogenic potentials in all the cassava samples (fermented and unfermented) were above 10mg HCN/kg, the value regarded by the Codex Alimentarius Commission of the FAO/WHO (1991) as safe. The FAO/WHO limit safe level can be achieved by heap fermentation of cassava roots with an initial cyanogenic potential of less than 32 mg HCN/kg and achievement of such low levels is applicable only if sweet varieties are used (Cardoso *et al.*, 2005). Bitter varieties with initial cyanogenic potentials in the range of 250 to 265 mg HCN/kg were used in this study. It has been suggested that the FAO/WHO (1991) cyanogenic potential safe level should be revised (Rosling, 1994). The human body, even with

very low protein intake, is able to detoxify 12.5 mg of cyanide every 24 hours. In a well nourished adult, the body can detoxify about 50 to 100 mg every 24 hours (Rosling, 1994). In a population were cassava is the main staple food, a basic daily energy need of 1500 kcal can be obtained from consumption of 500 g dry weight of cassava flour. Rosling (1994) suggested that cassava flour products with 25 mg HCN/kg may be eaten without any danger to human health. Indonesia has set the safe level of cyanogenic potential in cassava flour at 40 mg HCN/kg (Djazuli and Bradbury, 1999). The average cyanogenic potential value of 17 $\pm$ 6 mg HCN/kg obtained in this study after heap fermentation of bitter cassava roots for 4 days may be considered safe if the FAO/WHO (1991) safe level is revised according to Rosling (1994). However, it is important to develop further processing techniques to reduce cyanide such as a combination of grating of cassava roots, fermentation and sun drying or soaking of cassava roots in water and sun drying. Grating and crushing of cassava roots are very effective in removing cyanide because of the contact in the wet parenchyma between linamarin and the hydrolysing enzyme, linamarase (Westby, 2002, Cardoso *et al.*, 2005).

5.3 LABORATORY SIMULATION OF CASSAVA HEAP FERMENTATION USING ISOLATED MOULDS

Moulds grew and colonised the transversally and longitudinally cut parts of sliced cassava roots. Once the mould spores had produced mycelia, moulds were able to grow on the parts of cassava roots which had not been damaged. The mould spores took a long time to grow on parts of cassava roots where there was removal of cassava peel only.

This experiment supported the observation in the field, described in section 4.2.2, that mould mycelia appear first on damaged cassava roots and not on the peeled surface. *Rhizopus stolonifer* is incapable of penetrating through uninjured fruit surfaces. It can only enter through

wounds that occur during harvesting, transporting, post harvest treating and handling (Nishijima *et al.*, 1990).

Neurospora sitophila grew fast and rapidly covered the roots, but only slowly softened the cassava roots, while the *Rhizopus stolonifer* grew and covered the cassava roots slowly but softened the roots more rapidly than *Neurospora sitophila*. Essers *et al.* (1995b) also observed that *Rhizopus stolonifer* softened cassava roots more rapidly than *Neurospora sitophila*.

Slicing the cassava roots longitudinally increased the softness of cassava during the fermentation with moulds. Slicing the roots allowed the cassava to be softened rapidly, which is important because fermentation time was reduced. However, slicing of the roots, which has to be done manually, may be time consuming and a quicker method may need investigation such as introducing a manual chipping machine. Cassava roots softness occurs as a result of enzymatic cell wall degradation of cassava roots, enabling contact between linamarin and linamarase. The enzymes that degrade the cell wall are produced by moulds (Essers *et al.* 1995b).

5. 4 PROTEIN ENRICHMENT OF CASSAVA BY CO-FERMENTATION WITH COWPEA

5.4.1 Growth of *Rhizopus stolonifer* (*R. stolonifer*) and pH changes

Supplementation of cassava roots with cowpea prior to fermentation accelerated the growth of moulds, which is important because the time required for heap fermentation of cassava was reduced. The reduction of mould growth, observed after 48 hours of incubation, in cassava roots supplemented with cowpea at proportion 88:12 may be due to the low water activity in the mixture due to the high portion of dried cowpea flour in the mixture compared to the other

mixtures. The pH was in the range between 6 to 6.5, which is ideal for cyanogenic glucoside break down (White *et al.*, 1994).

5.4.2 Protein and ash content

The growth of *Rhizopus stolonifer* did not increase the protein content of cassava roots. The increase in protein content of flour prepared from fermented dried cassava roots was due to supplementation with cowpea. However, several studies have shown that cassava protein contents increased as result of solid state fermentation using various micro-organisms such as *Rhizopus oryzae*, *Aspergillus niger* and *Aspergillus oryzae* (Raimbault *et al.*, 1985; Daubresse *et al.*, 1987; Noomhorn *et al.*, 1992; Zvauya and Muzondo, 1993). As shown in tables 2.3, 2.4 and 2.5, the supplementation of cassava roots with cowpea increased the chemical score of essential amino acids of cassava roots, with the exception of sulphur amino acids, whose chemical score decreased with increase in amount of cowpea supplemented. Another advantage of co-fermenting cowpea with cassava is the reported reduction of anti nutritional factors in cowpea such as sugars responsible for flatulence (Sefa-Dedeh *et al.*, 2001; Lambot, 2002).

Supplementation of cassava with cowpea resulted in large increases in the protein content of the cassava flour, and the method of supplementation could easily be adapted at village level. The process would benefit areas where the cowpea is affordable and supplementation has to be carried out to a level where a product with acceptable sensory properties can be produced. Several studies have been carried out attempting to enrich cassava products with legumes such as cowpea and soybean (Oyewole and Aibor, 1992; Sanni and Sobamiwa, 1994; Mjimba, 1998). The results in this study are similar to those obtained by Oyewole and Aibor (1992) who obtained increases of protein in cassava flour (*fufu*) from 1.8% to 5.5% after cassava roots were supplemented with 20% of cowpea. In that work cassava roots

were spontaneously pre-fermented for 48 hours and partial water removed before mixing with cowpea.

The experimental protein values of cowpea supplemented cassava were higher than predicted theoretical values as the protein content of cassava roots was higher than the predicted.

The production of an unpleasant smell during fermentation may be an indication of the production, by moulds, of proteolytic enzymes, which degrade protein, liberating ammonia. The cause of the smell generated needs to be investigated to rule out possible production of toxic substances during the fermentation.

The increase of ash content during fermentation may be because starch is broken down by moulds to the volatile compounds, leading to a reduction in the overall weight of the cassava roots without any reduction of minerals that are constituents of ash.

5.5 EFFECT OF CO-FERMENTATION OF CASSAVA WITH COWPEA ON THE TEXTURE OF CASSAVA ROOTS AND VISCOSITY OF CASSAVA FLOUR PASTE

5.5.1 Growth of *Rhizopus stolonifer* and *Neurospora sitophila* and softness of the roots

Compared to the fermentation of cassava roots alone, the supplementation of cassava roots with cowpea resulted in rapid growth of moulds and softness of the roots. The fermentation time of cassava roots, which normally takes 72 hours to get sufficient softness, was reduced to 48 hours through cowpea supplementation. *Neurospora sitophila* grew faster than *Rhizopus stolonifer* but *Rhizopus stolonifer* softened the cassava roots more than *Neurospora sitophila*. A combination of the moulds may therefore contribute to a faster fermentation, attaining desirable softness of the cassava roots.

5.5.2 Viscosity of cassava flour paste

Fermentation of cassava roots by moulds up to 72 hours decreased the viscosity of cassava flour paste, which was taken to be an indication of the production of ∝-amylase by moulds. Supplementation of cassava roots with cowpea prior to fermentation reduced the viscosity of cassava flour paste. Acording to Sefa-Dedeh *et al.* (2000), cowpea fortification of cereal porridges increases the nutritional value and decreases the viscosity of the porridges. The viscosity of cassava flour paste, described as rubbery in texture, is higher than that of flour paste made from cereals. The rubbery characteristic in cassava paste is not favourable to the consumers (Essers *et al.*, 1995a). The reduction of the cassava flour paste viscosity by supplementing with cowpea may increase its acceptability.

The observed increase in the viscosity of cassava flour paste prepared from 24 hour fermented cassava roots supplemented with cowpea may be due to the decrease in sugars, which were being used by moulds during growth. Sugars reduce peak viscosity of starch (Whistler and Daniel, 1985).

5.6 SENSORY EVALUATION OF PASTE PREPARED USING FLOUR FROM CASSAVA ROOTS CO-FERMENTED WITH COWPEA

5.6.1 Appearance of *karakata*

Karakata prepared from unfermented cassava flour, especially laboratory prepared, had a white appearance that was found to be generally acceptable by panellists. *Karakata* from local unfermented flours was slightly grey probably due to the cassava roots at the rural area being sundried on a sandy soil surface and the dried roots being stored in a loft where they can become wet because of roof leakage. Both processes can promote undesirable mould growth

after cassava processing (Essers & Nout, 1989). Also, the dried roots stored in a loft get smoke from the fire usually being made in the houses. Supplementation of cassava roots with cowpea prior to fermentation improved the appearance of *karakata* prepared from fermented cassava flour.

5.6.2 Odour, Taste and Texture of *karakata*

The acceptability of the odour of *karakata* prepared from fermented cassava flour without cowpea supplementation was low. Fermentation of cassava roots supplemented with cowpea improves acceptability of aroma in *karakata,* although, during fermentation, the unpleasant smell was more intensive in cassava roots supplemented with cowpea than in cassava roots without cowpea supplementation. The flavour and texture of *karakata* samples prepared from cassava flours supplemented with cowpea were more acceptable than *karakata* from fermented cassava flours without cowpea supplementation. The acceptability of the flavour and texture of *karakata* is important because supplementation of cassava roots with cowpea prior to the fermentation gives a product of lower cyanogenic potential and higher protein content. Other studies have shown that cowpea flour can be used to partially replace wheat flour in wheat flour products with the purpose of increasing the nutritional value of the products. The results of the sensory evaluation of the quality of wheat flour products obtained were similar to products using 100% of wheat flour (McWatters *et al.*, 1995). In previous studies of enrichment of cassava flour by solid-state fermentation using selected fungi, sensory evaluation of the products was not carried out (Raimbault *et al.*, 1985; Daubresse *et al.*, 1987; Noomhorn *et al.*, 1992; Zvauya and Muzondo, 1993). Work done by Oyewole and Aibor (1992) on co-fermentation of cassava roots with cowpea resulted in cassava flour, *fufu*, with overall acceptability similar to the traditionally prepared *fufu*.

5.6.3 Cyanogenic potential

Supplementation of cassava roots with cowpea resulted in a product of lower cyanogenic potential due to some of the cassava being replaced by the legume. The cowpea supplemented product is thus more acceptable than the unsupplemented product.

6.0 CONCLUSIONS AND RECOMMENDATIONS

There were differences in the cyanogenic potential values of the cassava flours collected from different districts in Nampula Province, with the levels from heap fermented samples being generally significantly lower than those from sun-drying only. The level of cyanogens obtained after heap fermentation was higher than 10 mg HCN/kg, the level recommended by FAO/WHO (1991) as safe. Heap fermentation followed by sun drying does not adequately remove cyanide to safe levels; therefore, there is a need to improve the process to further reduce cyanide. Methods that use grating and crushing before fermentation are thus recommended, as they are effective in removing cyanide due to intimate contact between linamarin and linamarase (Cardoso *et al.*, 2005). Further investigation of heap fermentation needs to be done to achieve the FAO/WHO (1991) minimum recommended limit value such the optimization of the following conditions of heap fermentation: volume of the heap of cassava roots, the size of cassava roots chips, the heap container, duration of fermentation, etc. Results also indicate that the mean values of the cyanogenic potential of the flours collected from four districts in Nampula Province, Mozambique, were all above the WHO recommended value of 10 mg HCN/kg. There is need to investigate whether the WHO recommended limit in remote parts of Africa is actually practical as there have suggestions to change the limit (Rosling, 1994, Cliff *et al.*, 1999).

Moulds are the dominant microbes involved in the heap fermentation of cassava in Nampula Province, Mozambique. Inoculation of cassava roots with isolated moulds resulted in a shorter fermentation time compared to the natural fermentation under uncontrolled conditions. Further research is necessary to obtain controlled fermentation conditions, which can be easily adopted at rural household level. It would also be necessary to find ways of producing the moulds for inoculation in bulk using cost-effective growth media such as industrial waste. The role of bacteria and yeasts in the final product quality needs investigation. Laboratory simulation of cassava heap fermentation using isolated moulds showed that the fermentation reduces cyanogenic levels more than sun-drying alone. The protein content of cassava flour slightly increased with fermentation of the roots.

Neurospora sitophila grew faster than *Rhizopus stolonifer* on cassava roots under controlled conditions. *Rhizopus stolonifer* softened the cassava roots more than the *Neurospora sitophila*. A combination of the moulds would reduce the fermentation time. Slicing the cassava roots is recommended as it increased the rate of mould growth and softness of the roots during the fermentation. However, a quick method of slicing needs to be investigated as slicing the cassava roots manually would be labour intensive.

Fermentation of cassava roots with moulds resulted in a decrease in the viscosity of cassava flour paste, *karakata*. The less viscous paste is generally more acceptable than the more viscous paste. Therefore, controlled fermentation using moulds may increase acceptability of the paste.

Supplementation of cassava roots with cowpea resulted in large increases in the protein content of the flour produced and reduced fermentation time. Inoculated moulds grew well on cassava roots mixed with cowpea flour. The supplementation of cassava roots with cowpea flour prior to fermentation increased the growth rate of moulds, softness of cassava roots and decreased the viscosity of cassava flour paste. Depending on availability and cost of cowpea,

the supplementation of cassava roots with cowpea is highly recommended as it results in reduced fermentation time and a flour with highly increased protein content.

Sensory evaluation of *karakata* prepared from cassava flour supplemented with cowpea showed that the product was highly acceptable, even more acceptable than the unsupplemented product. The appearance, odour, taste and texture of the cowpea supplemented product were highly acceptable to the panellist. Cowpea supplementation of cassava roots prior to fermentation produces a product of lower cyanogen content and flour which can be used to prepare *karakata* with higher acceptability than unsupplemented cassava flour. Investigation into ways of carrying out the co-fermentation of cassava roots with cowpea under rural condition needs to be carried out. The possible growth of pathogenic microbes also needs investigation.

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