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 ± 20 to 90 ± 60 mg HCN/kg. The average total cyanogenic content for the unfermented samples $(64 \pm 60 \text{ mg HCN/kg})$ was significantly greater than that of the fermented samples $(34 \pm 30 \text{ mg HCN/kg})$.

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 ± 0.01 to 5.6 ± 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 ± 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 cofermentation of cassava roots with cowpea ($Vigna\ unguiculata$) using selected moulds. Cofermentation 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 ± 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

CONTENTS

4.0 NUTDODUCTION	Page
1.0 INTRODUCTION	
2.0 LITERATURE REVIEW	4
2.1 ORIGIN AND BOTANY OF CASSAVA	4
2.1.1 The origin and distribution of cassava	4
2.1.2 Taxonomy of cassava	4
2.1.3 Physiology and morphology of cassava	4
2.2 PRODUCTION AND IMPORTANCE OF CASSAVA	7
2.2.1 Ecology.	7
2.2.2 Cassava production.	7
2.2.3 Importance of cassava	7
2.3 CHEMICAL AND NUTRITIONAL COMPOSITION OF CASSAV	VA8
2.3.1 Chemical and nutritional composition of cassava roots	8
2.4 CYANOGENIC GLUCOSIDES	10
2.4.1 Cyanogenic glucosides in the plant kingdom	10
2.4.2 Distribution of cyanogenic glucosides in cassava plants	11
2.4.3 Biosynthesis of cyanogenic glucosides	11
2.4.4 Hydrolysis of cyanogenic glucosides	12
2.4.5 Toxicology of cyanogenic of cassava	13
2.4.6 Mechanisms of cyanogen detoxification by the human body	14
2.4.7 Methods for the analysis of cyanogenic glucosides	15
2.4.7.1 Titration method	16
2.5 CASSAVA PROCESSING	17
2.5.1 Cassava post harvest deterioration	17
2.5.2 Processing of cassava roots	18
2.5.2.1 Boiling	18
2.5.2.2 Sun drying	18
2.5.2.3 Fermentation	19

2.5.3 Biochemistry of fermentation of cassava roots and the role of ba	acteria and fungi 20
2.5.4 Mycotoxins in cassava products	22
2.5.5 Improvement of protein content of cassava products using micro	obial techniques 22
3.0 MATERIALS AND METHODS	27
3.1 CYANOGENIC POTENTIAL OF CASSAVA FLOUR SAMI	PLES COLLECTED
FROM SELECTED DISTRICTS OF NAMPULA PROVINCE, MO	OZAMBIQUE27
3.1.1 Study area	27
3.1.2 Sample collection	27
3.1.3 Analysis of cyanogenic potential of cassava flour samples	29
3.1.3.1 Preparation of assay reagents	29
3.1.3.2 Assay of enzyme activity	30
3.1.3.3 Extraction of cyanogens	30
3.1.3.4 Preparation of KCN Standard	30
3.1.3.5 Determination of cyanogenic potential	31
3.1.4 Moisture and dry matter content	32
3.1.5 Data treatment	32
3.2 MICROBIAL AND BIOCHEMICAL CHARACTERISTICS OF	F CASSAVA HEAP
FERMENTATION	32
3.2.1 Study area	32
3.2.2 Traditional heap fermentation	32
3.2.3 Enumeration of microbes	
3.2.4 Isolation of microbes	33
3.2.5 Identification of microorganisms	34
3.2.5.1 Identification of bacteria	34
3.2.5.2 Identification of moulds	34
3.2.6 Determination of cyanogenic potential	35
3.2.7 Determination of total crude protein	35
3.2.7.1. Preparation of Reagent	35
3.2.7.2 Digestion of sample	36
3.2.7.3 Distillation of digested sample	36
3.2.7.4 Titration of distillate samples	37
3.2.8 Determination of nH	37

3.3 LABORATORY SIMULATION OF CASSAVA HEAP FERM	ENTATION USING
ISOLATED MOULDS	37
3.3.1 Preparation of cassava	37
3.3.2 Preparation of mould spores	38
3.3.3 Fermentation of cassava slices	38
3.3.4 Description of mould growth	39
3.3.5 Texture analysis of cassava roots during fermentation	39
3.3.6 Determination of pH	39
3.4 PROTEIN ENRICHMENT OF CASSAVA BY CO-FERM	ENTATION WITH
COWPEA	40
3.4.1 Preparation of cassava roots and preparation of cowpea	40
3.4.2 Preparation of spores	40
3.4.3 Treatments of batches for fermentation	40
3.4.4 Biochemical analyses	41
3.4.4.1 Determination of ash content	41
3.4.5 Description of mould growth	43
3.4.6 Statistical analysis	43
3.5 EFFECT OF CO-FERMENTATION OF CASSAVA WITH OF TEXTURE OF CASSAVA ROOTS AND VISCOSITY OF CASSA	VA FLOUR PASTE
3.5.1 Preparation of spores	
3.5.2 Preparation of cassava roots and cowpea	43
3.5.3 Treatments of batches for fermentation	44
3.5.4 Description of mould growth	44
3.5.5 Texture analysis of cassava roots during fermentation	44
3.5.6 Determination of viscosity of cassava flour by "Hagberg Falling	g Number"45
3.5.7 Statistical analysis	45
3.6 SENSORY EVALUATION OF PASTE (KARAKATA) PREPAR	ED USING FLOUR
FROM CASSAVA ROOTS CO-FERMENTED WITH COWPEA	46
3.6.1 Samples used for sensory evaluation	46
3.6.2 Preparation of cassava flour paste (<i>karakata</i>)	47
3.6.3 Sensory evaluation of <i>karakata</i>	47

		VII
3.6.4 Dete	rmination of cyanogenic potential	48
3.6.5 Stati	stical analysis	48
4.0 RESULTS	S	49
4.1 CYANOGE	NIC POTENTIAL OF CASSAVA FLOUR COLLECTED FROM	
SELECTED	DISTRICTS OF NAMPULA PROVINCE, MOZAMBIQUE	49
4.1.1 Com	parison of processing methods in terms of cyanogenic potential	49
4.1.2 Com	parison of the cyanogenic potential of flour samples from four different	
loca	tions	54
4.2 MICROBIA	AL AND BIOCHEMICAL CHARACTERISTICS OF CASSAVA	HEAP
FERMENT.	ATION PROCESS	59
4.2.1 Trad	itional method of heap fermentation in Nampula Province, Mozambique .	59
4.2.2 Visu	al changes, temperature and pH in the heaps	59
4.2.3 Mici	obial analysis	61
4.2.4 Iden	tification of microorganisms	62
4.2.4	1.1 Lactic acid bacteria	62
4.2.4	1.2 Moulds	63
4.2.5 Cyar	nogenic potential and crude protein content of dry cassava chips	65
4.3 LABORAT	ORY SIMULATION OF CASSAVA HEAP FERMENTATION	USING
ISOLATED	MOULDS	65
4.3.1 Desc	cription of mould growth on different pieces of cassava root	65
4.3.2 Dete	rmination of cassava softness and pH	67
4.4 PROTEIN	ENRICHMENT OF CASSAVA BY CO-FERMENTATION	WITH
COWPEA		68
4.4.1 Desc	cription of mould growth	68
4.4.2 pH		69
4.4.3 Prote	ein content	70
4.4.4 Ash	content	73

RE OF CASSAVA ROOTS AND VISCOSITY OF CASSAVA FLOUR PAST	E.
	70
	78
RY EVALUATION OF PASTE PREPARED USING FLOUR FROM CASSAV	'A
CO-FERMENTED WITH COWPEA	81
Evaluation of the acceptability of the appearance of karakata prepared from	
ifferent cassava flour samples	31
Evaluation of the acceptability of the odour of karakata prepared from different	
assava flour samples	83
Evaluation of the acceptability of the taste of karakata prepared from different	
assava flour samples	35
Evaluation of the acceptability of the texture of karakata prepared from different	
assava flour samples	38
Cyanogenic potential	90
SSION)2
GENIC POTENTIAL OF CASSAVA FLOUR COLLECTED FROM	
TED DISTRICTS OF NAMPULA PROVINCE, MOZAMBIQUE	92
NIATION FROCESS	′4
ATORY SIMULATION OF CASSAVA HEAP FERMENTATION USING	
	96
ED MOULDS	70
	RE OF CASSAVA ROOTS AND VISCOSITY OF CASSAVA FLOUR PAST Growth of Rhizopus stolonifer and Neurospora sitophila Change in softness of cassava roots during fermentation Effect of mould fermentation and cowpea supplementation of cassava roots on the viscosity of cassava flour paste RY EVALUATION OF PASTE PREPARED USING FLOUR FROM CASSAV CO-FERMENTED WITH COWPEA Evaluation of the acceptability of the appearance of karakata prepared from different cassava flour samples Evaluation of the acceptability of the odour of karakata prepared from different cassava flour samples Evaluation of the acceptability of the taste of karakata prepared from different cassava flour samples Evaluation of the acceptability of the texture of karakata prepared from different cassava flour samples Evaluation of the acceptability of the texture of karakata prepared from different cassava flour samples Evaluation of the acceptability of the texture of karakata prepared from different cassava flour samples Evaluation of the acceptability of the texture of karakata prepared from different cassava flour samples Evaluation of the acceptability of the texture of karakata prepared from different cassava flour samples Scanage flo

	IA.
5.4.1 Growth of <i>R. stolonifer</i> and pH changes	97
5.4.2 Protein and ash content	98
5.5 EFFECT OF CO-FERMENTATION OF CASSAVA WITH COWPEA O	
TEXTURE OF CASSAVA ROOTS AND VISCOSITY OF CASSAVA FLOU	
5.5.1 Growth of <i>Rhizopus stolonifer</i> and <i>Neurospora sitophila</i> and softness of the	••••••
roots	99
5.5.2 Viscosity of cassava flour paste	100
5.6 SENSORY EVALUATION OF PASTE (KARAKATA) PREPARED FRO	OM CO-
FERMENTED OF CASSAVA ROOTS WITH COWPEA	100
5.6.1 Appearance of <i>karakata</i>	100
5.6.2 Odour, Taste and Texture of <i>karakata</i>	101
5.6.3 Cyanogenic potential	102
6.0 CONCLUSIONS AND RECOMMENDATIONS	102
7.0 REFERENCES	104

LIST OF TABLES

	Page
Table 2.1 Ch	emical composition of cassava roots and leaves
Table 2.2 Co	mparison of FAO protein amino acid patterns with amino-acid composition
of	protein of cassava and other foods
96 Table 2.4 Exp	pected amino acid balance when cassava:cowpea are mixed in proportion :4 g/g compared with cassava and cowpea alone
Table 2.5 Exp	28 g/g compared with cassava and cowpea alone
Table 3.1 A t	able showing assigned scores for various sensory attributes of <i>karakata</i> 47
Table 4.1 Cy	anogen contents of cassava flour from different districts classified according
to	processing method
Table 4.2 Tal	ble of pH, total mesophilic bacteria and lactic acid bacteria counts in fermented
cas	ssava roots, and cyanogenic potential and crude protein in dried fermented cassava
chi	ps after different periods of fermentation
Table 4.3 Sur	mmary of the characteristics of bacteria isolated from heap fermented cassava63
Table 4.4 Su	mmary of the characteristics of moulds isolated from heap fermented cassava roots
	64
Table 4.5 Mc	ould growth on different slices of cassava roots during fermentation at 30° C66
Table 4.6 Tex	xture of different slices of unfermented and fermented cassava roots67
Table 4.7 pH	changes during the fermentation of batches of cassava at 30 °C
Table 4.8 Gro	owth of Rhizopus stolonifer during fermentation of cassava and cassava + cowpea at
30°	°C69
_	changes during the fermentation of batches of cassava and cassava + cowpea 30 °C
	otal ash content of unfermented and fermented cassava flour prepared from
	ssava and cassava + cowpea
	rowth of <i>Rhizopus stolonifer</i> (CNF6) and <i>Neurospora sitophila</i> (CNF7) during
	mentation of cassava roots either supplemented with cowpea or not at 30° C76
	lentification of flavour of <i>karakata</i> by the panellist

LIST OF FIGURES

Page Figure 2.1 Cross section of a cassava storage root	
Figure 2.2 The generalized biosynthetic pathway for cyanogenic glucosides as proposed by	
Hahlbrock <i>et al.</i> (1968)	2
Figure 2.3 Hydrolysis of linamarin	
Figure 2.4 Metabolism of cyanide to thiocyanate in the human body	
Figure 3.1 Map of Nampula Province, Mozambique	
Figure 3.2 Sequence of experimental production of fermented cassava flour supplemented	J
with cowpea	2
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	1
by a combination of fermentation followed by sun-drying	2.
Figure 4.3 Distribution of cyanogenic potentials of samples of cassava flour processed by	_
sun-drying alone	3
Figure 4.4 Distribution of the cyanogenic potentials of cassava flour samples from Acordo	,
de Lusaka, Memba District.	5
Figure 4.5 Distribution of the cyanogenic potentials of cassava flour samples from Niyaro	
village, Monapo District	6
Figure 4.6 Distribution of the cyanogenic potentials of cassava flour samples from Quixaxe	Ŭ
village, Mogincual District.	7
Figure 4.7 Distribution of the cyanogenic potentials of cassava flour samples from Naconha	•
village, Mogovolas District.	8
Figure 4.8 Temperatures inside and outside the heaps during traditional fermentation,	_
measured in the morning	
Figure 4.9 Temperatures inside and outside the heaps during traditional fermentation,	
measured in the afternoon	
Figure 4.10 Changes in protein content during fermentation of cassava roots inoculated with	
Rhizopus stolonifer7	1
Figure 4.11 Changes in protein content during fermentation of cassava roots uninoculated72	
Figure 4.12 Protein contents of cassava flour supplemented with different quantities of cowpea	
flour	3
Figure 4.13 Hardness of cassava roots during fermentation with <i>Rhizopus stolonifer</i> and	
Neurospora sitophila7	8

Figure 4.14 Change in viscosity of cassava during fermentation with <i>Rhizopus stolonifer</i>	
and Neurospora sitophila.	80
Figure 4.15 General appearance acceptability scores of karakata prepared from different	
cassava flours	82
Figure 4.16 General odour acceptability scores of karakata prepared from different cassava	
flours.	84
Figure 4.17 General taste acceptability scores of karakata prepared from different cassava	
flours	86
Figure 4.18 General texture acceptability scores of karakata prepared from different cassava	
flours	89
Figure 4.19 Cyanogenic potential of different cassaya flours	91