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Heap fermentation of cassava (*Manihot Esculenta* Crantz) in Nampula province, Mozambique

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Abstract. Cassava (*Manihot esculenta* Crantz), a tropical root crop, is an essential part of the diet of the inhabitants of Nampula Province in Northern Mozambique. The root is mostly consumed after processing by heap fermentation and sun-drying. The study investigated the microbial and biochemical changes occurring during traditional heap fermentations in three households. The cassava was fermented over a period of 4 days. Temperature values at which extensive mould growth was observed ranged from 26 to 29°C. Lactic acid bacteria increased from 10^4 to 10^6 cfu/g during the fermentation. Isolates were identified as *Leuconostoc pseudomesenteroides*, *Leuconostoc mesenteroides*, *Enterococcus faecium* and *Weissella cibaria*. Moulds identified were *Neurospora sitophila* and *Rhizopus stolonifer*. The pH values of the cassavas decreased from 6.1 $\pm$ 0.01 to 5.6 $\pm$ 0.6 during heap fermentation. Average total cyanogenic levels in non-fermented cassava flour was 158 mg HCN/kg, while in fermented cassava flour, a value of 17 g HCN/kg was recorded. Protein concentration in the cassava flour slightly increased from 1.3 % to 1.8 % w/w dry matter during fermentation.

Introduction

Cassava (*Manihot esculenta* Crantz) is a very important crop in Mozambique, where it is the second most important staple food after maize and contributes 42 % of the total food energy (CIAT, 1990; Dahniya, 1994). Cassava is an essential part of the diet in Nampula Province, Mozambique and is mostly consumed after processing. The processing of the roots is mainly by sun drying (Zvauya *et al.*, 2002). Cassava flour is the mostly widely consumed cassava product in Nampula Province. The flour is made from chips that may be heap-fermented before sun drying or sun dried directly before milling into flour. Bitter varieties of cassava are normally fermented before sun drying and milling. The cassava flour obtained from fermented chips has a lower cyanogenic content than that obtained from non-fermented chips (Zvauya *et al.*, 2002). Non-fermented cassava has a low protein content, which remains relatively low in the fermented cassava chips (Essers *et al.*, 1995).

Very few studies have been carried out on the biochemical and microbial changes occurring during heap fermentation of cassava (Essers *et al.*, 1995). The fermentation is largely uncontrolled and results in variable quality products. A variety of microorganisms, including bacteria, yeasts and moulds are involved in the process, moulds being the most important (Essers *et al.*, 1995). The traditional heap fermentation process in Mozambique has not been studied previously. In this study, the biochemical and microbial changes occurring during heap fermentation of cassava were studied in Nampula Province, Mozambique. The predominant micro-organisms were isolated and identified.

Materials and Methods

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. The district is 3593 km^2 with about 86 000 inhabitants.

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. The fermentation processes were monitored for a period of 5 days from the onset until the end of fermentation where pH values were measured daily. Temperature was measured inside and outside the heap twice a day (morning and afternoon) throughout the fermentation. Samples were collected daily from the heaps for sun drying during 7 days at approximately 30 to 34° C. Afterwhich, cyanogens and crude proteins amounts were determined.

Enumeration of microbes. Samples (1 g) collected from the onset of fermentation until the end were placed into 9 ml peptone water (Oxoid, CM0009) and serial dilutions were made to 10^{-2} and 10^{-3}. Total aerobic mesophilic bacteria were determined on plate count agar (Oxoid, CM0325), lactic acid bacteria were determined using De Man-Rogosa-Sharpe (MRS) agar (Oxoid, CM0361) and yeasts and moulds were enumerated on Wort agar (Oxoid, CM0247). Cultures were incubated in air for 3 days at ambient temperature (30-32 °C).

Isolation of microbes. Further samples for microbial isolation and identification were collected from 3 localities: locality 1, situated 20 km West of Nacaroa Village; locality 2 in Nacaroa Village; locality 3 situated 20 km east of Nacaroa Village. Three family homesteads were visited in each locality. Samples of heap-fermented cassava roots at 1, 2 and 3 days of fermentation were collected from each family. In adition, dried cassava roots were collected.

Small pieces (1g) of fermented cassava collected from the heaps at different stages of fermentation were added to test tubes with 9 ml peptone water (Oxoid, CM0009). The contents of the test tubes were mixed by shaking and 0.1 ml of each suspension were then streaked over Wort agar (Oxoid, CM0247), Malt Extract agar (Oxoid, CM0059) and MRS agar (Oxoid, CM0361) plates. Cultures were incubated in air for three days at about 30° C. Predominant microbes were isolated and purified by repeated streaking of single colonies on the same media. The isolated microbes were preserved in 30% glycerol and stored at -80°C.

Identification of microorganisms. Bacteria were regenerated on APT agar (DIFCO 0654-17-0) by plating 0.1 ml of sample and incubating for 2 days at 30 °C. Cultures were observed under the microscope (1000x, Olympus, BH2). The bacteria were inoculated to APT broth (DIFCO 0655-17-9) and incubated at 30 °C. The final pH was measured after 48 h incubation at 30°C. The catalase test was done by emulsifying a loopful of growth from a plate with a drop of hydrogen peroxide (3%) on a slide and observing effervescence (Harrigan and McCance, 1966). For final bacterial isolates identification, RNA gene sequencing was used. Moulds were regenerated on Potato dextrose agar (Oxoid, CM0139) plates and their growth characteristics and reproductive structures were observed under the microscope (Olympus, BH2). A classification key was used for identification (Pitt & Hocking, 1997). Yeasts were also regenerated on potato dextrose agar (Oxoid, CM0139) but no attempt was made to identify the genera.

Determination of total cyanogen. Dry cassava pieces (5g) were placed into 10 ml of phosphoric acid (0.1 M) and stored at 4 °C until required. To extract cyanogens, the sample was homogenized and more 15 ml of 0.1M phosphoric acid added and later centrifuged for 10 min at 2500 rev min^{-1}.

Extracts were kept at 4°C until analysis. Total cyanogen in cassava samples was determined as described by Essers (1993). The enzyme, linamarase, used in total cyanogens assay was prepared from cassava latex as described by Haque and Bradbury (1999).

Determination of pH. Fermented cassava pieces (5 g) were suspended in 20 ml distilled water for pH measurement.

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

Results

Visual changes, temperature and pH in the heaps with time. 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 and the roots were 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, the inside temperature was lower than the outside temperatures (Figs. 1 and 2). The pH of the cassava roots slightly decreased during fermentation (Table 1), probably due to lactic acid production by lactic acid bacteria.

Microbial analysis. It was difficult to determine reliable counts of total mesophilic bacteria, yeast and moulds due to extensive mould growth on the plates. Total lactic acid bacteria increased from 10^4 to 10^6 cfu/g during the fermentation (Table 1).

Identification of microorganisms. For lactic acid bacteria determination, a total of 10 bacterial cultures were selected, three were cocci and seven were rods. Three were catalase positive and seven were catalase negative. The catalase negative strains were taken for identification. Five were identified as lactic acid bacteria: *Leuconostoc pseudo-mesenteroides* (CNB4), *Leuconostoc mesenteroides* (CNB6), *Enterococcus faecium* (CNB7) and *Weissella cibaria*

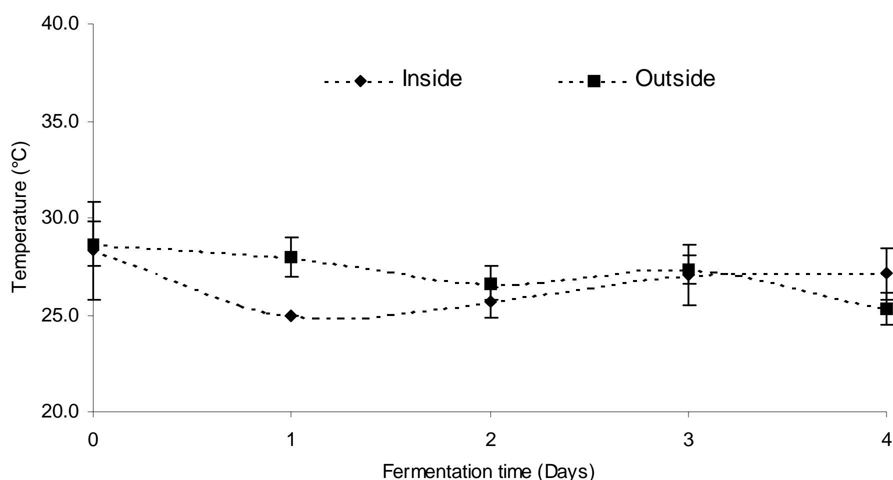

Figure 1: Temperatures inside and outside the heaps during traditional fermentation,measured in the morning (6.00 to 8.00 AM), n=3.

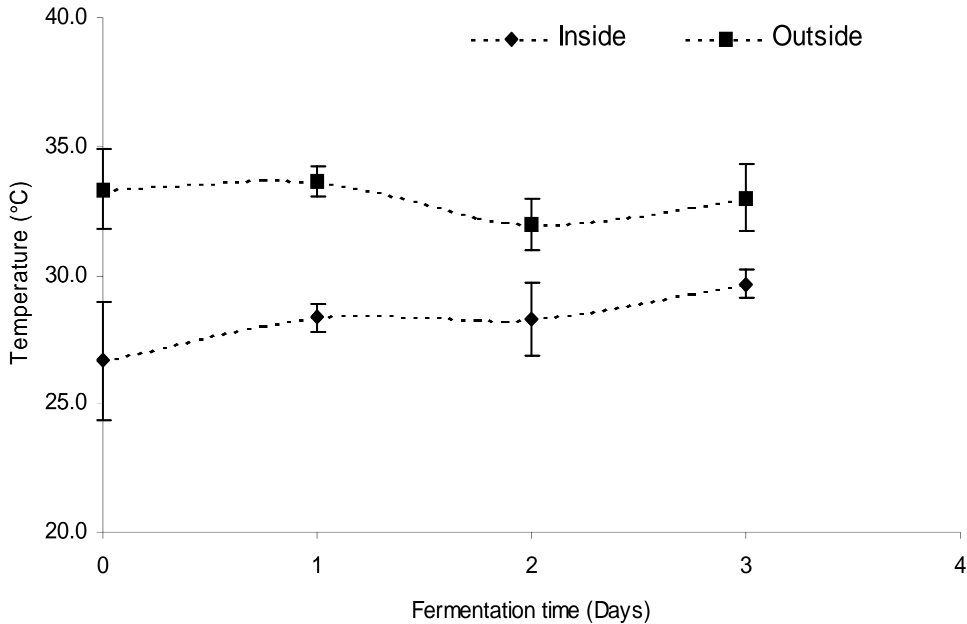

Figure 2: Temperatures inside and outside the heaps during traditional fermentation, measured in the afternoon (1.00 to 3.00 PM), n=3.

(CNB9) from cassava fermented. *Leuconostoc mesenteroides* (CNB10) was also isolated from dried cassava flour.

Moulds were observed in 2 to 3 day fermented cassava and in dry cassava. A black mould was dominant and grew in all the media used. Orange moulds were less evident on the fermented roots but grew well on media. The orange moulds were identified as *Neurosopora sitophila* (CNF1) and the black moulds as *Rhizopus stolonifer* (CNF6).

Cyanogens content of dry cassava chips. The cyanogens content in dried fermented cassava chips was 9 - 14% of that in dried unfermented chips (Table 1).

Protein content of dried cassava chips. The average protein content in dried cassava roots slightly increased from 1.3 % to 1.8 % (w/w) dry weight after 3 days of fermentation (Table 1).

Discussion

High ambient temperature makes local peasants use a place where there is usually shade during the day to create a lower temperature for fungal growth. The heap environment is also improved by covering the roots to avoid moisture loss. In Nampula, heap fermentation takes place during the dry season. The temperature inside the heaps was lower than the temperature outside during the fermentation. Evaporation of the moisture lowered the temperature inside the heap. Essers *et al* (1995) found that the temperature of the incubated roots was 2 to 12 °C high than the ambient temperature near the heaps, which was between 23 and 29 °C in the mornings.

The slight decrease of pH was probably caused by production of lactic acid by bacteria. The pH was maintained between 6 and 5, which is ideal for the glycoside break

Table 1: Resume of pH, Lactic acid count in fermented cassava roots and total cyanogens ant crude protein in dried fermented cassava chips after different periods of fermentation.

	Fermentation time (days)				
	0	1	2	3	4
pH fermented cassava root (n=3)	6.12 ± 0.01	6.11 ± 0.07	5.97 ± 0.37	5.59 ± 0.48	5.65 ± 0.64
Total Lactic acid bacteria in fermented cassava root (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 fermented cassava root (w/w) (n=3)	1.33 ± 0.12	1.42 ± 0.06	1.49 ± 0.28	1.80 ± 0.16	1.61 ± 0.08
Total cyanogens in dried fermented cassava root (mg HCN/Kg) (n=3)	158.27 ± 20.44	117.39 ± 24.75	29.83 ± 12.73	23.23 ± 10.61	16.86 ± 6.35

down (White *et al.*, 1994). The moulds, *Rhizopus stolonifer* and *Neurosopora sitophila* isolated here were also isolated and identified by Essers (1995). Moulds are the main microorganisms involved in heap fermentations. The fermentation plays an important role in reducing the residual total cyanogens in dried cassava chips. However, the cyanogens concentration in all the cassava samples (fermented and unfermented) were above the concentration (10mg/Kg) recommended by the Code Alimentarius Committee of the FAO/WHO (1991) as safe. Similar result were obtained in Uganda by Essers *et al.* (1995).

Conclusion

From the results obtained so far, it can be concluded that moulds are the dominant microbes involved in heap fermentation of cassava in Nampula Province, Mozambique. The fermentation reduces cyanogenic levels more than does sun-drying alone. The protein content of cassava roots slightly increased with fermentation.

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