Microbial load and microflora of cassava (*Manihot esculenta* Crantz) and effect of cassava juice on some food borne pathogens

Gulelat Desse, Mulugeta Taye ,
Debub University, Awassa College of Agriculture
P. O. Box 5 Awassa, Ethiopia

Abstract

The microbial load of cassava tubers that were grown at Awassa, Ethiopia, was analysed. The total viable microbial count of fresh cassava, before cleaning, ranged from $8.7x10^4$ to $2.1x10^9$ c.f.u/g whereas, in thoroughly cleaned product it was reduced to 10^6 c.f.u/g (% C.V., 19.5). *Enterobacteriacae* and spore former bacteria had mean counts of 10^4 and 10^3 c.f.u/g respectively. The dominant bacteria groups within the aerobic mesophillic flora were *Acinetobactor spp.* (29.1%), *Micrococcus spp.* (17.4%) and Enterbacteriacae (16%). Bacterial spores, *Pseudomonas, Moraxella* and *Aeromonas* spp. Were detected in a small proportions. Fate of *Staphylococcus aureus, Bacillus cereus* and *Listeria monocytogenes* in cassava juice was also evaluated. Except in *B. cereus* the growth of the bacterial strains was retarded only at higher concentrations.

Introduction

Cassava is a major source of carbohydrate particularly for low-income consumers. It grows in a wide range of climatic conditions and yields better than most other crops under drought conditions (Cock, 1985). At present, cassava is a major source of food for 800 million people (Nweke, 1996). It has also been used as a food in some parts of Ethiopia, particularly in the south and southwestern regions for a century (Taye, 1994).

Information on the microbiology or nutritional quality of food items in Ethiopia are limited (Gashe *et al.* 1982; Gifawosen nad Bisrat, 1982; Gashe; 1985, 1987; Ashenafi, 1994). There are very few reports on the microbial quality and fate of the various food borne pathogens during the storage of cassava cultivars in Ethiopia.

The level of micro organisms in food items is essential as some of them are hazardous to health. For instance, if the number of *Bacillus cereus* and *Staphylococcus aureus* exceeds 106 cfu/ml, it is suspected of causing food poisoning (Geopfert, *et al.* 1972; Tatini, *et al.* 1973) and *Listeria monocytogenes* causes menengitism abortion and prenatal septicaemia mainly in new born, infants and immuno suppressed adults (Gray and Killinger, 1966).

The purpose of this study was, therefore, to evaluate the microbial load of fresh cassava and the behaviour of *B. cereus*, *S. aureus* and *L. monocytogenes* in cassava juice.

Materials and Methods

Sampling

A total of 120 matured fresh tuber roots of Amarokello Red and Umbure cultivars were collected from the experimental site of Awassa College of Agriculture, Awassa. Samples were put in sterile containers, immediately brought to the laboratory and processed for the following microbiological parameters.

Microbiological analysis: 25 gm of sample was aseptically removed and homogenised in 225 ml sterile 0.1% peptone water using lab blender for ten minutes.

Aerobic mesophillic bacteria: samples were further diluted in sterile tap water and volumes of 0.1 ml of appropriate dilutions were spread-plated in duplicates on pre-dried surfaces of plate count agar (PC; Merck). Colonies were counted after incubation at 30 to 32°C for 48 h.

Staphlococci: To identify *Staphylococcus aureus* appropriate dilutions were spread-plated in duplicate plates of Manitol Salt Agar (Oxoid) and incubated at 30 to 32° C for 48-72 h. Ten colonies from countable plates were picked and slide and tube coagulase test was done.

Enterbactriaceae: From the appropriate dilutions 0.1 ml volumes were taken, spread-plated in duplicates onto Violet Red Glucose agar (Oxoid) plates and incubated at 30 to 32° C for 24 h.

Bacterial spores: 0.1 ml of appropriate dilutions (after heating the sample for fifteen minutes) in a water bath of 80° C) were spread-plated in duplicates on the surface of PC (Merck) and colonies were counted after incubation at 30 to 32° C for 24 h.

Yeasts and Molds: Volumes of 0.1 ml appropriate dilutions were spread-plated in duplicates in pre-dried surfaces of Chloramphenicol-Bromophenol-Blue agar (CBB), (consisting of g/l in distilled water: yeast extract, 5.0; glucose, 20.0; chloramphenicol, 0.1; Bromophenol Blue, 0.01; agar, 15). The pH was adjusted to 6.0 to 6.4 yeast colonies were counted after incubating the plates at 25-27° C for 5 days.

Flora assessment: After colony counting, 10-15 colonies were selected at random from countable PC agar plates. Sub-cultures were further purified by repeated plating and a total of 700 strains were isolated and tentatively differentiated into various bacterial groups by the following characteristics: Phase contrast microscopy was used to examine cell shape and grouping, presence or absence of endospores and motility; Gram reaction was determined using the KOH test of Gregerson (Gregerson, 1978); cytochrome oxidase was tested by the method of Kovacs (Kovacs, 1956); catalase test was made with 3% (v/v) H_2O_2 solution; and glucose metabolism was investigated by the O/F test of Hugh and Leifson (Hugh and Leifson, 1953).

Preparation of cassava juice: juice from cassava was extracted by fruit grinding centrifugal machine and steam-sterilized. The juice was diluted in Brain Heart Infusion (BHI) Broth (Merck) to give final

concentrations of 25%, 50% and 75% Cassava juice. 100% was considered as undiluted juice and BHI broth served as a control. An electrode of a pH meter was dipped into the juice to measure its pH.

Test Organisms: *Baccilus cereus* (WS1537), *Staphylococcus aureus* (WS 1759) and *Listeria monocytogenes* (WS 2300) were obtained from the culture collections of Bacterologosches Institu, SVA, Weihenstephan, Germany.

Inoculation of cassava juice with test strains: For the purpose, cultures (overnight) of the test strains were separately inoculated in the various concentrations of the juice and the control tubes having a final innoculum level of 10^2 - 10^3 cfu/ml. After mixing thoroughly, the tubes were incubated at 30 - 32° C for 8 hours.

Analysis of samples: Appropriate dilutions from the freshly inoculated tubes were surface plated on Brian Heart Infusion Agar (MERCK), in duplicate to determine the initial innoculum level. All the tubes were then sampled aseptically after four and eight hours. The media used were: Manitol Slat agar for S. aureus, Modified McBride agar for L. monocytogenes and Baccilus cereus agara for B. cereus. All these media are from Oxoid and inoculated plates were then incubated at 32°C for 24-48h after which colonies were counted.

Results

The total viable microbial count of un-cleaned cassava ranges from $8.7x10^4$ to $2.1x10^9$ cfu/g. After a thorough cleaning with a chlorinated tap water, however, the count reduced to 10^5 cfu/g (**table 1**). From the various bacterial groups isolated from well-cleaned cassava, *Staphylococcus spp.*, yeasts and molds were detected in a very low proportion (<10^3 and 10^2 cfu/g respectively), whereas, plate count agar and Violet Red Bile Glucose agar yielded relatively higher microbial counts. The mean counts of AMB were around 10^6 cfu/g with variation among samples (C.V., 19.6%). *Enterobacteriacae* and Bacterial spores had mean counts of 10^4 and 10^3 cfu/g respectively. Marked variations, however, were noted in the counts of Bacterial spores (C.V., 29.5%). Isolates from the plate count agar yielded different bacterial genera of epidemiological significance at varying frequencies (**Table 2**). The dominant ones were *Acinetobacter spp.*, 29.1%; *Micrococcus spp.*, 17.4% and *Enterobacteriacae*, 16%. *Acinetobacter, Micrococcus,* and *Enterobacteiacae* were isolated from 65.8%, 46.7% and 34.1% of the samples respectively.

The pH value of cassava juice was found

Table 1. Microbial load (log/gm) of Cassava

Bacterial groups	X± S.D	%CV
AMB	6.62 ± 1.31	19.5
Staphylococcus	2.19 ± 0.22	10.0
Enterobactriacae	4.29 ± 0.86	19.6
Bacterial spores	2.84 ± 0.84	29.5
Yeasts and moldsq	1.33 ± 0.38	29.3

X= Mean; S.D.= Standard Deviation; CV= Coefficient of variation

Table 2. Frequency distributed of dominant AMB of Cassava

Bacterial groups	Positive samples		Isolates	
	No.	%	No.	%
Acinetobacter spp.	79	65.8	204	29.1
Micrococcus spp.	56	46.1	122	17.4
Enterobactreaceae	41	34.1	112	16.0
Pseudomonas spp.	26	21.7	90	12.9
Bacillus spp	21	17.5	74	10.6
Moraxella	17	14.2	36	5.2
Aeromonas	12	10.0	19	2.72

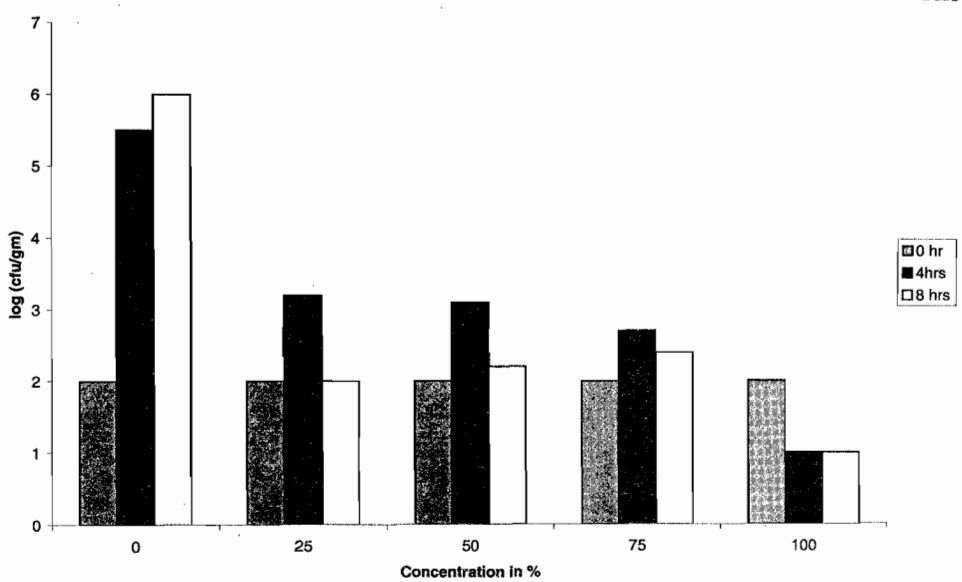

Fig. 1. Fate of L. monocytogens at different concentrations of cassava juice

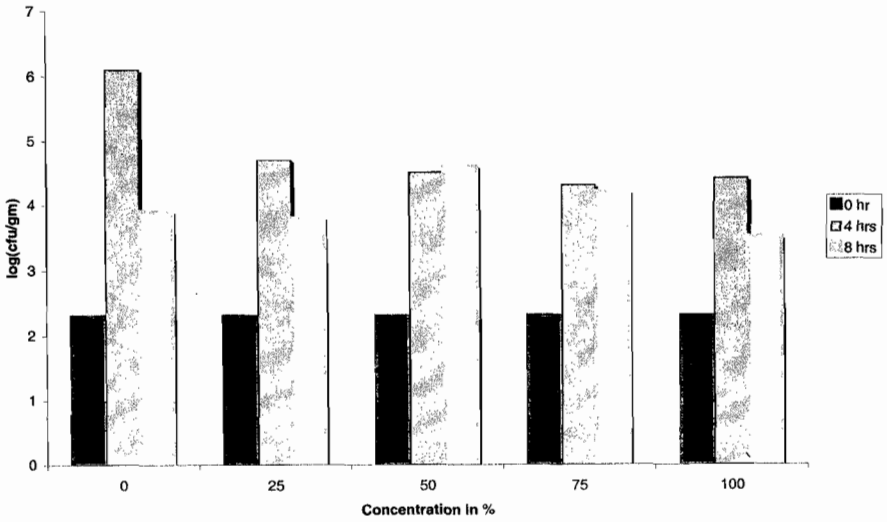

Fig. 2. Fate of S. aureus at different concentrations of cassava juice

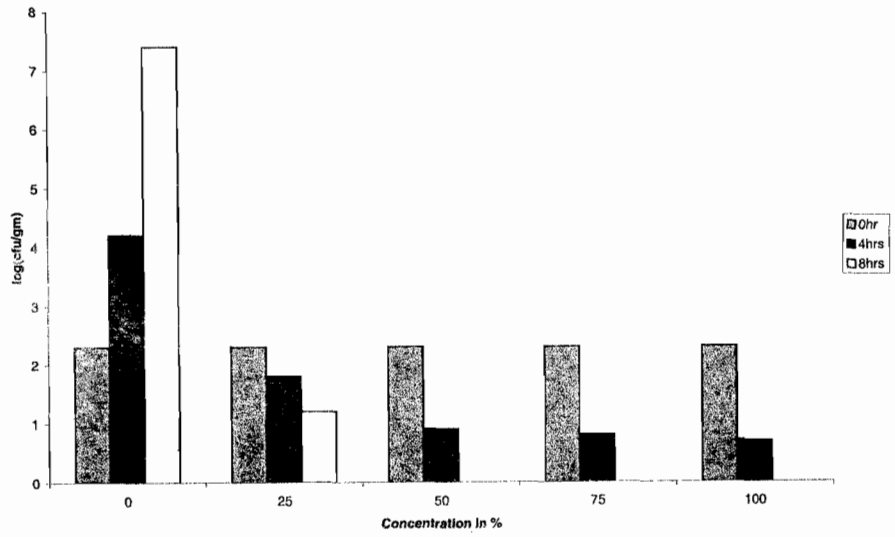

Fig. 3. Fate of B. cereus at different concentrations of cassava juice

to be 6.3. In the media containing no cassava juice all the test strains grew without any retardation. At 50% concentration the growth of *L. monocytogenes* was affected and complete inhibition was observed at 100% concentration. Concerning *B. cereus*, its growth was completely inhibited at concentrations of 25%, whereas, with increasing concentrations counts for *S. aureus* decreased.

Discussion

Coryneforms, lactic acid bacteria, Sporeformers, coliforms, micrococci and pseudomonas are saprophytes that were observed in most vegetables and tubers (Geopfert, 1980). The microflora of cassava growing in Ethiopia may, thus, be considered saprophytes, which usually are found in fresh vegetables. As cassava tubers are not properly stored here in this country, the bacteria load and its multiplication should not be considered acceptable. The cassava samples are found to consist *staphylococcus*, that may cause staphycoccal food poisoning and it was reported that production of enterotoxin occurs at *Staph. Aureus* count of 106 cfu/g (Tatini, *et al.* 1970). If the level of contamination with B. cereus exceeds 10^6 cfu/g, it can play a role in the transmission of *B. creus* food poisoning (protnoy, *et al.*, 1976).

L. monocytogenes is ubiquitous in nature (Farber, *et al.* 1987). There is a possibility of contamination of cassava with *L. monocytogenes* and this results in abortion in pregnant women, menengitis and encephalitis in immunocompromised hosts (McLaughlin, 1987).

The high bacterial load of cassava observed in this study is, therefore, due to its unhygienic handling, which as a result, may initiate spoilage bacteria to multiply. As is shown in table 1, counts of the various bacterial groups from well cleaned cassava is low. However, the potential of their multiplication makes it hazardous for consumption. Among the AMB, Acinetobacter is found to be the dominant one. Although, the growth failed to continue during storage trials, an insignificant amount of Salmonella, only in some of the fresh samples, was detected in this study. However, some reports (Yoo, *et al.* 1983; Frank and Bayer, 1984) have shown that there is an incidence of salmonella in many food items like fish and egg. It is, therefore, important that everything likely to come into contact with the cassava is clean. Moreoyer, the natural process is irreversible ad preservation principle should be employed to slow down the deterioration, hence increasing the overall quality and storage shelf life of the product. The sooner any preventive measures are taken after harvest, the

greater the chance of reducing post-harvest losses.

Wastage of food due to poor handling practices of food products in Ethiopia is extreme. On the other hand, low income and the low level of technology in the country has given no chance to the people to use suitable ways of packing and storage. Hence famine, nutritional deficiencies, rural-urban migration and mortality rate may be minimised if proper health education is given and improved traditional food preservation techniques are widely introduced. These foods should be handled hygienically to minimise contamination and avoid unnecessary loss. This study, thus, indicates that the shelf life of cassava product can be improved from microbiological point of view and that cassava juice may not have an inhibitory effect on Pathogens, which may contaminate the tubers.

Acknowledgement

The technical assistance of Amsalu Gobena and Wondosen Tadesse is highly acknowledged.

References

Ashenafi, M. 1994. Microbial flora and some chemical properties of ersho, a starter or tef (Eragrostis tef) fermentation. *World journal of Microbiology and Biotechnology.*

Cock. 1985. A new potential for neglected crop. West view press, Booder, Co., USA.

Frank HK, Bayer I. 1984. Effects of natural and sterilised pepper on the Spoilage rate of ready to eat soups. Deutsche Lebensmittel Rundeschau 80,369-374.

Gashe, B.A. Girma, M. and Bisrat, A. 1982 Tef fermentation. The role of Microorganisms in fermentation and their effect on the nitrogen content of tef. SINET, *Ethiopian journal of Science* 5, 21-25.

Gashe, B.A. 1985. Involvement of lactic acid Bacteria in the fermentation of tef (Erogrostis tef), an Ethiopian fermented food. *Journal of food science* 50, 800-801.

Gashe, A.B. 1987. Kotcho fermentation. *Journal of Applied Bacteriology* 62, 473-477.

Geopfert JM; Spira WM, Kim HU, 1972. Bacillus cereus: Food poisoning organism. A review. *J. Milk Food Tech*; 35,213-27.

Geopfert JM, 1980. Vegetables, Fruits, nuts and their products. In: Microbial Ecology of Foods. (ed. JH Silliker). ICMSF. New York.

Gifawossen, C. and Bisrat, A. 1982. Yeast flora of fermenting Teff (Ergrosts tef) dough. SINET, *Ethiopian Journal of Sciences*; 5, 21-25.

Gray ML., Killinger AH. 1966. Listeria monocytogenes and listeric infections. Bacteriol. Rev; 30: 309-82.

Gregerson G. 1978. Rapid method for distinction of Gram negative from gram positive bacteria. European *J. Appl. Microbiol.* 5:123-127.

Huh R, Leifson E. 1953. The taxonomic significance of fermentative versus oxidative gram negative bacteria. *J Bacteriol.* 178:703.

Kovacs N. 1956. Identification of Pseudomonas pyocyanae by the oxidate reaction.

Nature 66, 24-26.

Mclaughlin J. 1987. *Listeria Monocytogenes*, recent advances in the taxonomy and epidemiology of listeriosis in humans. *J. Appl. Bacteriology*; 63: 1-11.

Mogessie Ashenafi. 1989. Microbial load, incidence and antibiotic resistance of some disease causing microorganisms on row food items consumed in Ethiopia. Mircen *J. Appl. Microbial. Biotechnol.,*: 5;313-319.

Nwekw, F.I. 1996. Cassava processing in sub-Sahara Africa: Implications for expanding cassava production. IITA Research. International Institute of Tropical Agriculture. Bulletin, pp7-13.

Portnoy BL, Goepfert JM and Harmons SM. 1976. An outbreak of Bacillus cereus food poisoning resulting from contaminated vegetable sprouts. Am *Epidemiol;* 103:589-594.

Tatini SR, Wasela WD, Jezeski JJ, Morris HA. 1973. Production of Staphylococcal enterotoxin A in blue, break, Mozerrella and Swiss cheeses. *J Dairy Science*; 56, 429-35.

Taye Mulugeta. 1994. Cassava in southern and south western Ethiopia. Cassava news letter, vol 18, pp 6-7, CIAT, Columbia.

Yoo YJ, Lee HC, Min BY, Shin DH. 1983. Microbial studies of foods. *Korean Journal of Applied Microbiology and Bioengineering*. 11, 131-136.