Original article

Microbial load and microflora of "chat" (*Cata edulis* Forsk) and effect of "chat" juice on some foodborne pathogens

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Abstract: Chewable leaves of "chat" (*Cata edulis*) were collected from retail shops in Awassa and analyzed for their microbial load and microflora in March - June, 1995. Aerobic mesophilic bacteria, Enterobacteriaceae and bacterial spores had mean counts of >104 cfu/g. *Staphylococcus* sp., *Bacillus* sp. and yeasts and molds had mean counts of 103 cfu/g. Between 60% and 80% of the "chat" samples yielded Enterobacteriaceae, *Bacillus* sp., *Micrococcus* sp. and *Staphylococcus* sp. The aerobic mesophilic flora was dominated by Enterobacteriaceae (25%), *Bacillus* sp. (22%), *Micrococcus* sp. (18%) and *Staphylococcus* sp. (13%). About 47% of the *Staphylococcus* isolates were *Staphylococcus aureus.* "Chat" juice retarded growth of *Salmonella typhimurium, Salmonella enteritidis* and *Staphylococcus aureus* at higher concentrations, but complete inhibition was not attained even at 100% concentration. *Bacillus cereus* was inhibited at lower concentrations (25%). Inhibition of *Listeria monocytogenes* was observed only at 100% concentrations. [*Ethiop. J. Health Dev.* 1997;11(1):83-87]

Introduction

"Chat" (*Cata edulis*) is an evergreen shrub of the family Celastracae that grows primarily in Ethiopia, Kenya and Yemen. In the literature, "chat" is consistently referred to as khat and is socially and economically one of the most important plants not only of many countries of Eastern and Southern Africa but also of the Middle East (1). Only the fresh leaves have the desired effect and the "chat" habit has, thus, remained endemic to these areas (2). Due to the development of international air travel, "chat" use has spread to countries far away from the areas of cultivation (2). The juices of the fresh leaves are ingested and this produces central stimulation in man. Between five and ten million people are reported to chew "chat" (2).

In Ethiopia and neighboring countries, "chat" is commonly consumed for social recreation (3) and for other purposes by various occupational groups and students (4). It is also chewed for religious and medicinal uses (5).

There are various studies on the chemical and pharmacological nature of "chat" (2,6). Studies on clinical effects of "chat" have shown that "chat" chewing results in significant increase in blood pressure and the medical and psychological effects are hazardous both to the individual and the community (7).

In Ethiopia, "chat" is consumed without any pre-treatment. Considering the non-hygienic transportation and wrapping processes of this plant from farm-hand to distributors, whole sellers, retailers and finally the consumer, "chat" is exposed to various sources of contamination. It may, thus, be contaminated by a variety of microorganisms including those that can cause food-borne

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diseases. The purpose of this study was, therefore, to determine the microbial load and microflora of "chat" and evaluate if the "chat" juice could eliminate some important food-borne pathogens.

Methods

Collection of Samples: A total of 51 fresh "chat" samples were collected in sterile plastic bags from different "chat" retail markets in Awassa, Ethiopia. The samples were immediately brought to the laboratory and processed for the following microbiological parameters.

Microbiological Analyses: Ten grams of fresh chat leaves were mixed with 90 ml of sterile deionized water in a sterile screw cap bottle and shaken manually for over two minutes to obtain a homogenized washed sample suspensions.

Aerobic mesophilic bacteria: Samples were further diluted in sterile water and volumes of 0.1 ml of appropriate dilutions were spread-plated in duplicate on pre-dried surfaces of Plate Count Agar (PC; Merck) with a bent glass rod. Colonies were counted after incubation at 30 to 32oC for 48 h.
Enterobacteriaceae: Volume of 0.1 ml of appropriate dilutions were spread plated in duplicate on pre-dried surfaces of Violet Red Bile Glucose Agar (Oxoid) plates. The plates were incubated at 30 to 32oC for 24 h.

Bacillus cereus: Volumes of 0.1 ml of appropriate dilutions were spread plated in duplicates on pre-dried surfaces of *Bacillus cereus* agar (Oxoid) and colonies were counted after incubation at 30 to 32oC for 24 h.

Staphylococci: Appropriate dilutions were spread-plated on duplicate plates of Mannitol Salt Agar (Oxoid) and incubated at 30 to 32oC for 48-72 h. Ten colonies from countable plates were picked and slide and tube coagulase test was done to identify *Staphylococcus aureus*.

Bacterial spores: Tubes containing 10 ml of suspensions were heat shocked in a water bath at 80 oC for 15 min. A volume of 0.1 ml of the pasteurized sub-samples was streak plated on pre-dried surfaces of Plate Count Agar and incubated at 30-32oC for 48 h.

Yeasts and molds: Volume of 0.1 ml of appropriate dilutions were spread-plated in duplicate on 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; pH, 6.0 to 6.4. Yeast colonies were counted after incubating the plates at 25-27oC for 5 d.

Flora assessment: After colony counting, 10 to 15 colonies were selected at random from countable PC agar plates. The sub-cultures were further purified by repeated plating. A total of 532 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 Gregersen (8); cytochrome oxidase was tested by the method of Kovacs (9); catalase test was made with 3% (v/v) H2O2 solution; and glucose metabolism was investigated by the O/F test of Hugh & Leifson (10).

Preparation of "Chat" Juice: Juice from fresh consumable "chat" leaves was extracted by fruit grinding centrifugal machine and steam-sterilized. As preliminary observations had indicated that filter-sterilized and steam- sterilized juice had no noticeable difference in activity on test strains, steam sterilized juice was used in subsequent experiments. The juice was diluted in Brain Heart Infusion (BHI) Broth (Merck) to give final concentrations of 25%, 50% and 75% "chat" juice. Undiluted juice was 100% and BHI broth without "chat" juice served as a control.

Table 1: **Microbial load (log cfu/g) of fresh chewable "chat" leaves.**

Bacterial groups	x ± S.D.	%C.V.
AMB	5.86 ± 0.82	14
Bacterial spores	4.79 ± 0.91	19
Enterobacteriaceae	4.04 ± 1.25	31
Staphylococcus sp.	3.35 ± 0.84	25
Bacillus cereus	2.19 ± 0.22	10
Yeasts and molds	3.19 ± 0.61	19

AMB, Aerobic mesophilic bacteria x, Mean

S.D., Standard deviationC.V., Coefficient of variation

The pH of the juice was measured by dipping an electrode of a pH meter into the juice.

Test Organisms: The following bacterial test strains were used in this study. *Salmonella typhimurium* (A 13), *Salmonella enteritidis* (A 2), *Staphylococcus aureus* (WS 1759), *Bacillus cereus* (WS 1537) and *Listeria monocytogenes* (WS 2300). The cultures were obtained from the culture collections of Bakteriologisches Institut, SVFA, Weihenstephan, Federal Republic of Germany.

Table 2: **Frequency distribution of dominant aerobic mesophilic bacteria from fresh chewable "chat".**

	Positive samples		Isolates	
Bacterial groups	No.	%	No.	%
Enterobacteriaceae	41	80.4	133	25.0
Bacillus sp.	35	68.6	115	21.6
Micrococcus sp.	38	74.5	97	18.2
Staphylococcus sp.	32	62.7	68	12.8
Coryneforms	51.0	37	7.0	
Aeromonas sp.	19	37.3	20	3.8
Pseudomonas sp.	12	23.5	18	3.4
Streptococcus sp.	8	15.7	16	3.0
Lactobacillus sp.	10	19.6	15	2.8
Acinetobacter sp.	8	15.7	13	2.4

Inoculation of "chat" juice with test strains : Overnight cultures of the test strains were separately inoculated in the various concentrations of "chat" juice and the control tubes to give a final inoculum level of 102 - 103 cfu/ml. The inoculated tubes were mixed thoroughly and incubated at 30-32oC for 8 h.

Analyses of samples: Appropriate dilutions from freshly inoculated tubes were surface plated on Brain Heart Infusion agar (MERCK) in duplicate to determine the initial inoculum level. The various tubes were then sampled after four and eight hours. Inoculated tubes were sampled (1 ml) aseptically and appropriate dilutions were spread-plated on the following media (all from OXOID). XLD agar for *Salmonella*, Mannitol Salt agar for *Staph. aureus*, Modified McBride agar for *L. monocytogenes* and *Bacillus cereus* agar for *B. cereus.* Inoculated plates were incubated at 32oC for 24-48h for colony counting. **Results**

Of the 51 "chat" samples, 29 had aerobic mesophilic counts of >1.0x106 cfu/g. The mean count was 7.2 x 105 cfu/g with little variation in counts among samples (C.V., 14%). Enterobacteriaceae and bacterial spores had mean counts of around 104 cfu/g and *Staphylococcus* sp., *Bacillus cereus*, and yeasts and molds had counts of 103 cfu/g. Marked variations were noted in counts of Enterobacteriaceae and *Staphylococcus* sp. among samples (C.V., 25%) (Table 1).

The aerobic mesophilic flora consisted of a variety of microorganisms (Table 2) and the dominant ones were Enterobacteriaceae (25%), *Bacillus* sp. (22%), *Micrococcus* sp. (18%) and *Staphylococcus* sp. (13%). Enterobacteriaceae, *Micrococcus* sp., *Bacillus* sp. and *Staphylococcus* sp. were isolated from 80%, 75%, 69% and 63% of the "chat" samples, respectively.

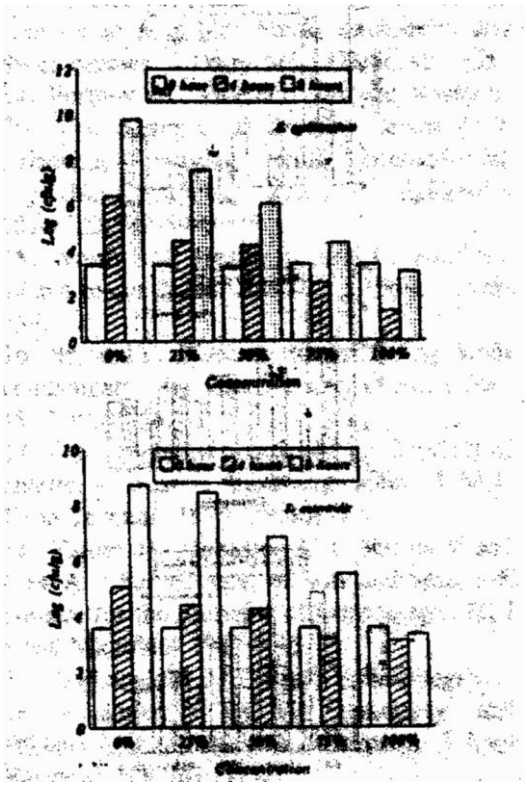

Figure 1: **Fate of *S. typhimurium* and *S. enteritidis* at different concentrations of "chat" juice.**

About 47% of the *Staphylococcus* isolates were found to be *Staphylococcus aureus* and the remaining were coagulase negative staphylococci.

"Chat" juice had pH value of 6.2. All test strains grew luxuriously in media containing no "chat" juice. Retardation of our *Salmonella* test strains increased with increasing concentrations. However, only decrease in count was noted at 100% concentration (Figure 1).

B. cereus was completely inhibited at concentrations of 25%. Maximum counts for *Staph. aureus* decreased with increasing concentrations. However, no marked difference was noted in initial and final counts at 100% concentration. *L. monocytogenes* growth was affected at 50% and higher concentrations with complete inhibition noted at 100% concentration (Figure 2).

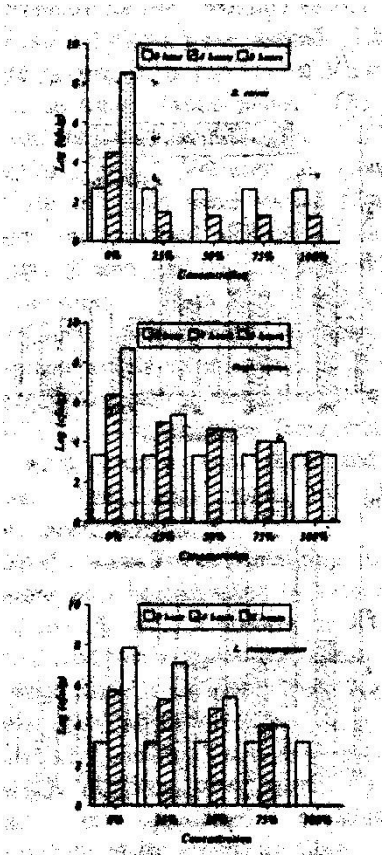

Figure 2: **Fate of *B. cereus, Staph. aureus,* and *L. monocytogenes* at different concentrations of "chat" juice**.

Discussion

The initial microflora of most edible plant leaves comes from air, insects, animals, soil and water. The activity of man, such as cultivation by hand, will introduce and/or distribute microorganisms into ecological niches from which they were previously absent. Finally, the introduction of human and other animal waste material into the water or soil will have an obvious impact on the flora of vegetables (11).

Studies have shown that most of the organisms on fresh vegetables are saprophytes such as coryneforms, lactic acid bacteria, spore formers, coliforms, micrococci and pseudomonads (11). The microflora of "chat" may thus be considered saprophytes that are usually found on many other fresh vegetables.

In comparison to bacterial counts of vegetables upon arrival to a processing plant (12), "chat" leaves have much lower bacterial counts. However, as "chat" leaves are consumed without further cleaning or other treatment, their bacterial load should not be considered acceptable. Considering the fact that "chat" leaves had mean counts of 104 cfu/g for *Staphylococcus*, 47% of which were *Staph. aureus*, the leaves may possibly result in Staphylococcal food poisoning. Enterotoxin production can occur at *Staph. aureus* count of 106 cfu/g (13). The presence of *B. cereus* in "chat" leaves, though at lower levels, may indicate that "chat" can play a role in the transmission of *B. cereus* food poisoning if level of contamination exceeds 106 cfu/g. An outbreak of *B. cereus* food poisoning due to home-nurtured vegetable sprouts has been reported (14).

The "chat" plant is usually cultivated around the homesteads and is highly likely that human and animal wastes are used as fertilizers. Various types of infections such as shigellosis, typhoid fever, cholera, amebiasis (15), outbreaks of salmonellosis (16) and infectious hepatitis (17) are reported to be caused by raw vegetables where "night soil" was used as fertilizers. Considering the ubiquitous nature of *L. monocytogenes* (18), contamination of "chat" leaves with *L. monocytogenes* is possible. *L. monocytogenes* causes abortion in pregnant women and meningitis and encephalitis particularly in immunocompromised hosts (19).

"Chat" consumption can, thus, be considered hazardous to health and the ingestion of "chat" juice may not have an inhibitory effect on pathogens which may contaminate the leaves.

A decrease in the microbial load of chat leaves may be achieved through a thorough washing of the leaves before chewing.

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