Original article

Microbial load and incidence of Staphylococcus aureus in market Bulla and Kotcho, traditional Ethiopian processed food products from Enset

(Ensete ventricosum)

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Abstract: Thirty samples each of bulla and kotcho, processed products of enset (Ensete ventricosum) were collected from the Awassa open market and analyzed for some microbiological and biochemical properties. Both products had high counts of aerobic mesophilic bacteria and yeasts (≥106 cfu/g). Coliform counts were markedly higher in bulla (10^5 cfu/g) than in Kotcho (around 103 cfu/g). Counts of enterococci was also high in both food types (10^4 to 1Q' cfu/g). Micrococcus and Bqcillus species dominated the aerobic flora in both products. All samples yielded staphylococci at levels > $1.0x10^5$ cfu/g and Staphylococcus au reus constituted between 50 and 100% of the isolates. Bulla and Kotcho yielded various yeast species and Rhodotornla glutinis, Kluyveromyces marxianus and Pichia membranefaciens were isolated from most or all samples. All products had pH values around neutral and moisture contents of around 50% .Bulla and Kotcho appeared to be processed in unhygienic conditions. Unfermented bulla and kotcho are likely to pose health problems and could spoil easily. [Ethiop. I. Health Dev. 1996;10(2):117-122]

Introduction

Enset (Ensete ventricosum), designated as false banana due to its physical resemblance to the banana plant, is cultivated in the densely populated areas of south and southwest Ethiopia and is a staple food for around 17 % of the Ethiopian population. The plant is also a source of feed for livestock and fiber for making various products (1) .The leaves are frequently used as packaging materials. The agronomic properties of the plant ate reported by several workers elsewhere (2-4) .The plant flowers at an age of 4-5 years, but is usually harvested before flowering and before the stored starch is used up by the plant for vegetative reproduction. Harvesting in the

Department, Awassa College of Agriculture, Awassa, Ethiopia areas surrounding Awassa is from the end of
October until January (5).

Processing mainly consists of decortication, pulverization, shredding, fermentation and squeezing. Processed enset has various vernacular names but the terms bulla and kotcho are the most common. Bulla is a white powder of enset extracted from the pulp after mashing and squeezing. The I1\ass of the pulp is buried in a pit and fermented into kotcho. A 4-S year old enset plant yields about 26 to 42 kg of enset flour. One person is estimated to consume an average of.;430- 700 g per day (6).

There are various ways of cooking enset products (S). Preparation of porridge from bulla and steam-cooked pancake from kotcho are among the common ones in many parts of the country .In areas surrounding Awasa,

maize and enset products constituted S4% and 4S % , respectively, of the total main dishes (S) . Food composition values of bulla and kotcho as reported by Agren and Gibson (7) i-ndicate that the products are high in carbohydrate and low in protein and fat (Table 1)

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Table 1: Food composition of bulla and kotcho in terms of 100g edible portion

	Bulla	**Kotcho**
Food energy (Kcal)	200	186
Moisture (%)	48.7	53.5
Protein (g)	0.6	0.5
Fat (g)	0.3	0.4
Carbohydrates (mg)	49.0	44.8
Calcium (mg)	82	70
Phosphorus (mg)	36	45
Iron (mg)	3.7	7.9

Information on the microbiology of enset products is scanty .There are few studies on the important microorganisms responsible for the fermentation of kotcho (8) and spoilage of kotcho (9). f3ut these studies were based on village fermentation techniques in the areas surrounding Addis Ababa. No information is available on the microbiology of enset fermentation or on the microbiological quality of the finished products in the southern part of Ethiopia. The aim of this study was to evaluate the microbiological quality of the two important enset products as made available to the consumer in the Awassa market.

Methods

Source and Collection of Samples. This study was carried but in Awassa. It is located 276 Km south of Addis Ababa and serves as the capital city of Southern Ethiopia Administrative Region. It has a population of .about 63,000. A total of 30 bulla and 30 kotcho samples were collected at random from all vendors from an open market on different sampling days. The samples were niicrobiologically analysed within two h of collection.

Microbiological analyses. Twenty-five g of bulla and kotcho samples were separately blended in 225 ml of sterile water using a Stomacher Lab Blender (Model 400, Seward, JAC, London, UK) and processed for the following microbiological tests.

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 32°C for 28 h.

Coliforms: Volume of 0.1 ml of appropriate dilutions were spread plated in duplicate on pre-dried surfaces of Violet Red Bile Agar (Oxoid) plates. The plates were incubated at 30 to 32°C for 24 h. Pink colonies with bile precipitation around them were counted as coliforms.

Bacterial spores: Portions of four ml and 0.4 ml from the 1: 10 homogenates were added to two different bottles containing 40 ml of molten PCA and heat shocked in a water bath at 80°C for 10 min. The contents of each bottle were then distributed between two petrldishes and incubated at 30-32°C for 48 h. Staphylococci: Appropriate dilutions were spread plated on duplicate plates of Mannitol Salt Agar (Oxoid) and incubated at 30 to 32°C for 48 h. Twenty colonies from countable plates were picked. Slide and tube coagulase test were done to identify Staphylococcus aureus .Five samples each of bulla and kotcho were used for this purpose.

Enterococci: Appropriate dilutions were spread plated on duplicate plates of Kanamycin Aesculin Azide agar (Oxoid) and incubated at 30 to 32°C for 48h.

Yeasts and molds: Volumes 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 25-27°C 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 720 strains were isolated and only 666 could be 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 (10); cytochrome oxidase was tested by the
method of Kovacs (11); catalase test was made with 3% (v/v) H2O2 solution; and glucose metabolism was investigated by the O/f test of Hugh and Leifson (12).
Yeast isolates from bulla and kotcho samples were characterized based on their fermentation of and gas production in glucose, galactose, sucrose, maltose, lactose, raffinose according to the methods of farkas (13). Assimilation of the above sugars and nitrate assimilation was tested by the auxanographic method of Barnett et al. (14).

Determination of pH: pH of was measured by placing the electrode of a digital pH meter in 1/5 dilution of the samples.

Determination of moisture content: Moisture content was determined by drying a sample to constant weight in an oven.

Table 2: Mean counts (log cfu/g) of various bacterial groups in bulla and kotcho

	pH	**I**	**II**	**III**	**IV**	**V**
Bkulla						
X	6.9	7.64	3.00	4.66	5.04	6.36

S	0.12	0.90	0.66	0.91	1.15	0.62
%CV	1.7	11.8	22.1	19.5	22.8	9.8
Korxho						
X	6.9	8.07	3.51	4.99	2.93	6.50
S	0.1	8.87	0.89	0.98	1.84	0.67
%CV	1.5	10.7	25.2	19.7	62.6	10.3

Results

The counts of aerobic mesophilic bacteria and yeasts in bulla and kotcho were high ($\geq 10^6$ cfu/g) with little variation in counts among samples (coefficient of variation, C.V., 9-10%) (Table 2). Coliform count, however, was markedly higher in bulla than in kotcho although significant variations were noted within samples with C.V. values of 22,8% and 62.2%, respectively. Aerobic spore formers and enterococci had lower counts in both food types with significant variations in counts within samples (C, V " ca, 20%)

The pH value of all bulla and kotcho samples was near neutral (6.9) and no significant variation was observed within samples (C,V .<2%).

A large number of strains were isolated from bulla and kotcho samples and the isolates on both food types were dominated by Micrococcus spp. followed by Bacillus and Staphylococcus spp. The Gram negative isolates constituted a low proportion of the dominant microorganisms in both food types (Table 3),

Table 3: Percent distribution of dominant isolates in bulla and Kotcho

Food type	**Mean AMC**	**Number of isolates**					
			I	**II**	**III**	**IV**	**V**
Bulla	$4.37*10^7$	339	57	13	26	2	2
Kotcho	$2.90*10^8$	327	66	11	17	6	-

Micrococcus spp. were among the dominant isolates in all bulla and most kotcho samples (Table 4). Around half of the bulla samples were also partly dominant by Staphylococcus and Bacillus spp. Bacillus spp. were also part of the dominant flora in half of the kotcho samples. The Gram negatives constituted part of the dominant flora in only few samples.

Table 4: Frequency of isolation of dominant bacteria from bulla and kotcho

Isolate	**Bulla (30)**	**Kotcho(30)**
Micrococcus	30	27
Staphylococcus	14	9
Bacillus	14	16
Alcaligenes	3	6
Enterobacteriaceae	3	0

Staphylococci were isolated from all bulla and kotcho samples and had counts of > $1.0xl0^5$ cfu/g (Table 5). All samples yielded Staphylococcus aureus. and it constituted 50 to 100% of the isolates.

Bulla and Kotcho samples yielded various yeast species at varying frequencies (Table 6). Rhodotorola glutinis was isolated from all bulla and kotcho samples, and over half of them yielded *Kluyveromyces marxianus* and *Pichia membranefaciens* .

Bulla and kotcho contained around 50% moisture with no marked difference within samples (Table 7).

Table 5: Counts of staphylococci in bulla and kotcho samples and proportion of S.aureus

	Staphylococci (cfu/g)	**S. aureus (% of isolates)**
B-1	$1.1*10^5$	70
B-2	$2.6*10^5$	69
B-3	$9.5*10^5$	51
B-4	$1.4*10^5$	79
B-5	$1.8*10^5$	100
B-1	$2.3*10^5$	61
B-2	$3.2*10^5$	69
B-3	$3.1*10^5$	87
B-4	$2.4*10^5$	100
B-5	$2.9*10^5$	76

Table 6: Yeast species isolated from bulla and kotcho.

	Frequency of isolation (%)	
	Bulla	**Kotcho**
Rhodotorula glutinis	100	100
Kluyveromyces marxianus	67	67
Pichia membranefaciens	67	67
Debaromyces rouxii	25	25
Zygosaccharomyces rouxii	30	30
Saccharomyces cervisiae	32	32
Candida kueusii	30	30

Table 7: Moisture content of bulla and kotcho

Range			**SD**	**%CV**
Bulla	41.84-54.19%	49.03%	6.42	13
Kotcho	42.43-55.04%	48.50%	4.67	10

Discussion

The high count of aerobic mesophilic bacteria observed in bulla and kotcho could be the result of frequent hand contact during decortication, pulverization and squeezing. The pits and the utensils used for collection could also be major sources of contamination. The fact that the Gram positive bacteria, particularly Micrococcus and Bacillus spp. , Were dominant indicated that hand contact, utensils and soil are the most important sources of contamihation. The dominance of the Bacillus species in the two products may also be considered as health concern in possible B. cereus food poisoning. In a few cases the Gram negative bacteria constituted a small part of the dominant flora. These could have been associated with the decaying plant material. However, the high counts of coliforms in bulla and enterococci in bulla and kotcho may also be indicative of the poor hygienic conditions during processing.

Bulla and kotcho, as fermented products, should normally have lower pH values. Various reports indicated that these products have pH values of around 4 (8,9). Our samples, collected from all vendors on different sampling days, however, invariably had pH values around neutral. This indicated that the bulla and kotcho products that are brought to the Awassa market are not fermented. Producers of these products around Awassa keep the fermented products for local consumption or an important occasion, and unfermented ones are used for immediate income generation purposes (Personal communication). Products with neutral pH and a considerable amount of moisture are likely to allow proliferation of pathogenic or spoilage microorganisms This may also explain the high coliform counts and dominance of aerobic spore formers in bulla and kotcho which normally are inhibited in acidic conditions (15-18).

The isolation of *Staphylococcus* aureus from all bulla and kotcho samples poses a considerable health hazard. S. aureus is known to produce a variety of heat stable enterotoxins which may still be potent after baking of the products. S. au reus was reported to produce enterotoxins at levels higher than 10^{6} cfu/g (19,20). Our S. aureus counts of 10^{6}-106 cfu/g were not far from the minimum reported so far .Since S. aureus was reported to withstand the adverse effects of low pH (21) and could grow at pH values as low as 4.6 (22), fermentation alone may not guarantee elimination of S. aureus from bulla and kotcho. Therefore, care should be made to avoid contamination in the initial and subsequent stages of processing.

A variety of yeast species were isolated from all bulla and kotcho samples and could have originated from the plant itself. Since most of our yeast isolates utilize only simpler fermentable sugars, they may not result in any significant change in the chemical composition of the products as the products consist mainly of starch. However, proteolytic and lipolytic species of Klyuveromyces and Candida could result in loss of the small amount of protein and fat contained in bulla and kotcho .

Bulla and kotcho sold at the Awassa open market were not in the fermented condition and were of unsatisfactory microbiological quality. The various traditional processing stages are open to contamination by spoilage or pathogenic microorganisms. Thus an appropriate temperature/time relationship must be determined for the baking or cooking of the products that can guarantee elimination or reduction of pathogens and enterotoxins to a safe level.

Acknowledgement

The technical assistance of Haile Alemayehu and Tsigereda Bekele is acknowledged.

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