ORIGINAL ARTICLE

MICROBIOLOGICAL SAFETY OF STREET VENDED AYIB IN JIMMA TOWN, SOUTHWEST ETHIOPIA

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ABSTRACT

The wide spread habit of street vended cheese consumption is a potential cause for food borne illness, besides the common factors such as over-crowding, inadequate sanitation, and poor hygiene. Therefore, the aim of this study was to assess the microbial quality of street vended Ayib and the associated health risks to consumers of the product in Jimma Town, south west Ethiopia. The microbial quality of different sample of Ayib being sold on the streets of Jimma Town was assessed following the standard microbiological analysis from December, 2010 to June, 2011. Accordingly, a total of 60 samples were collected from three streets Ayib selling market sites of Jimma Town for the analysis of Aerobic Mesophilic Bacteria (AMB) a, Enterobacteriacea, Staphylococcus, Lactic Acid Bacteria, Yeasts and Molds. Preliminary survey was also conducted to get information on the handling practice to trace source of contamination. Moreover, physico-chemical characteristics such as pH and titratable acidity of the Ayib were determined. Data was analyzed using SPSS of windows version 16.0. The mean microbial count of samples was found to be dominated by both AMB and Lactic Acid Bacteria, followed by Enterobacteriacea. The pH and titratable acidity were 4.2±0.02 and 0.21, respectively, indicating the Ayib samples were in acidic range. From among a total of 198 isolates characterized, only 13.1% were found to be Gram negative bacteria with the likelihood that some are potentially pathogenic. Even though the handling practice of Ayib sellers was very poor, the acidic nature of the food somehow provided protection against survival and growth of pathogenic microorganisms. However, bodies concerned and sanitary workers should work on health education in relation to Ayib hygiene before the likely outbreak of Ayib-borne diseases.

Key words/Phrases: ***Ayib, LAB, Microbial safety, physicochemical parameters, Street food***

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INTRODUCTION

Cheese is the curd or hard substance formed by the coagulation of milk of certain mammals. The preparation of cheese probably dates back to the time when nomadic tribes of Eastern Mediterranean countries carried milk of domesticated mammals in sacks made from animal skins or gourds or in vessels such as stomachs or bladders (Helen and Elisabeth, 1990). Cheese is made from sour milk by means of concentrating raw milk or fermentation through acid preparation (Donnely, 2004). Usually, fermentation of cheese at small scale is natural, without defined starter cultures used to initiate the process (Mogessie Ashenafi, 2002). Thus, the microbial load and type in cheese can vary from sample to sample.

Milk is an excellent medium for growth of microorganisms that could be introduced during collection, storage, transportation and processing, as it provides rich nutrients for microbes, (about 79% water, 15% protein, 2-7% fat, 1% ash, and 3% soluble milk constituents), and has neutral pH (Pacheco and Galindo, 2010). However, fermentation of raw milk discourages the growth of some pathogenic microbes as the media turns into acidic. Lactic acid bacteria (LAB), are naturally present in raw milk of healthy animal (Walstra *et al*., 2005), are likely the prime agents in producing soured (fermented) milk (Pacheco and Galindo, 2010). The majority of the strains belong to the genera *Lactoccoccus, Lactobacillus, Leuconostoc* and *Enterococcus* are designated as a starter flora responsible for acid development during cheese manufacturing (Pacheco and Galindo, 2010). Naturally, fermented milk is known to have more stable, helpful to health (Fernandez *et al.*, 1987), and has extended shelf-life besides improving the microbial quality and safety of the final product. However, the safety of cheese with respect to food-borne diseases is of great concern around the world. This is mainly true in developing countries, where production of milk and various dairy products often takes place under unsanitary conditions.

Generally, different microorganisms such as which contributed in preservation of cheese and production of desirable flavor and physical characteristics, and pathogens or their toxin may constitute health hazards and spoilage microorganisms can exists in raw milk cheese (Pacheco and Galindo, 2010). However, data on the microbiological quality and safety of ready-to-eat local cheese in Ethiopia is scanty (Mogessie, 1990). Therefore, this study was designed to assess the microbial quality and safety of Ayib sold on streets of Jimma Town, South West Ethiopia.

MATERIALS AND METHODS

Study site and period

The study was conducted in Jimma Town, located 345km southwest of Addis Ababa, the capital city, with latitude of $07^{0}39''$, longitude of $36^{o}50''$ and altitude ranging from 1700-1750 meter above sea level. The study was conducted from December, 2010 to June, 2011.

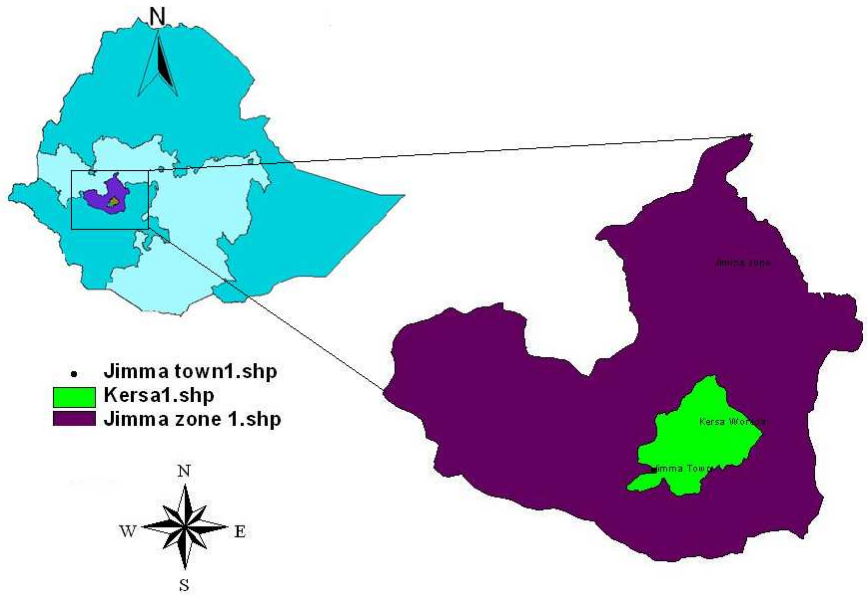

Figure 1 Map of the study site

Sample collection and preparation

A total of 60 Ayib samples were collected from different street markets found in Jimma Town and transported to laboratory for microbial analysis using ice box. Structured interview was conducted by the researcher to sellers while collecting Ayib samples to obtain preliminary information about the care taken at household level including how they store the Ayib until they brought it to market as well as the quality of materials with which they transport.

Samples (250gm) of Ayib were collected in sterile flask (500ml) separately and transported to laboratory at Jimma University, Department of Biology, Microbiology and Biomedical Sciences Laboratory. Samples were processed within an hour after collection. Twenty five grams of the Ayib sample was separately drawn and blended in 225ml of sterile physiological saline solution (0.85% NaCl). The samples were homogenized and appropriate dilutions were spread plated in duplicates on pre-dried surfaces of respective media for microbial count: aerobic mesophilic bacteria (AMB) were counted on Plate Count Agar (PCA) (Oxoid, UK) after incubation at 32 ^{o}C for 48 hours; *Enterobacteriaceae* were counted on MacConkey agar (Oxoid, UK) after incubated at 32^{o}C for 48 hours. Pink to red purple colonies with or without haloes of precipitation were counted as member of *Enterobacteriaceae*. Staphylococci were counted on Mannitol Salt agar (MSA, Oxoid, UK) after incubation at 32^{o}C for 48 hours. Lactic acid bacteria (LAB) were counted on MRS (De Mann Rogossa and Sharp) agar (Oxoid, UK) after incubated at 30-32^{o}C under anaerobic condition using anaerobic jars for 48hrs. All snow white colonies were counted as LAB. Similarly, Yeasts and molds were counted on Sabouraud agar plus 0.1g chloramphenicol incubated at 25-28^{o}C for 2-5 days. Smooth (non-hairy) colonies without extension at periphery (margin) were counted as yeasts. Hairy colonies with extension at periphery were counted as molds.

After enumeration, 10- 15 colonies were randomly picked from countable plates of PCA, MSA, MRS, and MacConkey Agar plates and further purified by repeated plating on nutrient agar and broth. Cell morphology and arrangement, Gram reaction, colony characterization and broth features were determined following standard microbiological methods: KOH test, a test for lipopolysaccharide, was made to distinguish between gram-positive and gram-negative bacteria (Gregersen, 1978). Catalase test was performed by adding few drops of 3% H_2O_2 on plates of an overnight culture of the pure isolates (MacFaddin, 1980). Cytochrome oxidase test and O/F test (oxidative or fermentative utilization of glucose) were conducted following standard microbiological methods (Hugh and Leifson, 1953).

pH was measured using digital pH meter (NIG. 333, Naina solar LTD, India) after homogenizing 10gm of the Ayib in 90 ml of distilled sterile water (Erkmen and Bozkurt, 2004). Titratable acidity was determined according to the method used by Antony and Chanrda (1997). Briefly, about 10 gm of Ayib sample was mixed in distilled water (20ml) and filtered through whatmann No.1 filter paper. About 2-5 drops of 0.5% phenolphthalein indicator was added to 20ml of the filtrate as indicator and titrated against 0.05M NaOH to the end point of phenolphthalein. Titratable acidity was expressed as g lactic acid/100g of juice and calculated using the following formula:

$$TA = \frac{MNaOH \times ml\ NaOH \times 0.09 \times 100}{ml\ juice\ sample}$$

Where, TA = titratable acidity; MNaOH = Molarity of NaOH used; ml NaOH =

amount (in ml) of NaOH used; 0.09 = equivalent weight of lactic acid

Data analysis

Microbiological and physico-chemical data were analyzed using SPSS soft ware (version 16). The significances of differences among mean values were computed using one-way ANOVA. Significances were considered at P-value less than 0.05 ($P<0.05$).

Ethical consideration

Data was collected up on verbal consent of Ayib handlers. Official letter of permission to collect data was obtained from Jimma University, Department of Biology. Interview was also administered by personal willingness of Ayib sellers and producers.

RESULTS AND DISCUSSION

Processing and handling of Ayib

The preliminary information collected from Ayib handlers showed some unhygienic activities of handlers and indicated the likelihood of Ayib contamination. Since all interviewed Ayib vendors (100%) had used *enset* or Musa leaves for covering and spice to improve the flavor of the Ayib, the practice revealed high possibility of contamination of Ayib with any of microbes linked with the leaves and traditional spices. Moreover, about 91.6% of Ayib producers used gourd for temporary storage of Ayib and well water for Ayib processing (Table 1). Since all Ayib producers did not have vocational training on food processing and handling, they all followed traditional processing procedures which don't involve application of any aseptic techniques. Besides, their knowledge on Ayib borne disease was almost none.

Table -1 Ayib processing, handling, transportation, and knowledge of Ayib borne diseases by Ayib handlers in Jimma town, February, 2011

Description	No of respondents	%
Ayib processing equipment and strong time		
▪ in gourd for 1-3 days	16	66.6
▪ > 3 days	8	33.33
▪ clay pot	0	00
Use of spice		
▪ yes	24	100
▪ no	-	-
Source of cleaning water		
▪ well	22	91.66
▪ tap	2	8.33
Temporary shortage		
▪ ground	22	91.66
▪ clay pot	2	8.33
Ayib quality identification method		
Color	36	100
Flavor	36	100
Method of showing to customers		
Opening container	36	100
▪ flavor	36	100
▪ allowing test	36	100
Transportation materials to the market		
▪ clay pot	-	-
▪ enset/Musa leaf	60	100
Knowledge of Ayib born disease		
▪ yes	-	-
▪ no	60	100
Any access to performance training		
▪ yes	-	-
▪ no	60	100

Beside direct contamination via processing, cross-contamination of the product due to unhygienic condition such as handling with other raw foods, from shelves, racks, or storage equipment, handling area or from general environment can be possible (Khan and Rockliff, 2002). Moreover, initial microfloras of the raw milk are also crucial in determination of cheese safety and qualify (Anon, 2007). For example *E. coli* O157: H7, often transmitted to humans via unpasteurized milk, can survive for extended periods of time in several types of acidic foods e.g. cheese and yogurt (Anon, 2007). In general the permitted levels of potentially pathogenic microbes for all cheese type are set in Australia New Zealand Food Standards Code (FSANZ). Accordingly, for all cheese type the count of *E. coli* and *Staphylococcus*/g of sample must not exceed 10 and 10^2 CFU/g respectively (Khan and Rockliff, 2002). In line with this specification, count of S*taphylococcus* observed in this study

was much higher (5.39 log CFU/ml) than the standard. Cheese is generally considered a potentially hazardous product as it is normally intended to be undercooked and eaten as purchased without further processing. Especially if the milk has not been pasteurized, it is difficult to ensure the safety of the cheese no matter how good the control of hygiene during production.

Physico-chemical characteristics of Ayib

The mean pH and titratable acidity of Ayib samples examined were 4.2±0.02 and 0.21 respectively (Table 2). The mean pH observed in this study was comparable with similar sample analyzed from the same country, Awassa, southern Ethiopia (Mogessie Ashenafi, 1990). As the media is found in acidic range, it can support some acid tolerant bacteria and fungus. According to U.S. food and Drug administration, the principal pathogenic microbes of concern associated to milk and processed milk are *Salmonella* spp, *L. monocytogens*, *S. aureus*, and pathogenic *E. coli*. However, as the pH of media became acidic their growth rate severely affected and even some of them (*L. monocytogens*) cannot survive when the pH dropped to ≤ 5.5 (Lado and Yusuf, 2007).

Table 2 pH and titratable acidity of street sold Ayib in Jimma town, February, 2011

Physic- chemical characteristics	**pH**		**TA**
	Mean	Range	Mean
	4.2+0.02	3.2 - 4.9	0.21

Microbial count

Ayib samples were found dominated by almost the same load of AMB (6.13 log CFU/ml), LAB (6.0l log CFU/ml), and followed by Enterobacteriacae (5.96 log CFU/ml). The mean counts of molds (5.84 log CFU/ml) and yeast (5.5 log CFU/ml) were also higher (Figure 1). The low pH of Ayib analyzed did not inhibit the growth of acid tolerant yeasts and molds rather allowed their proliferation. However, a high yeast count in low pH moisture cheese is not a matter of concern to the safety (Benkerroum and Tamine, 2004).

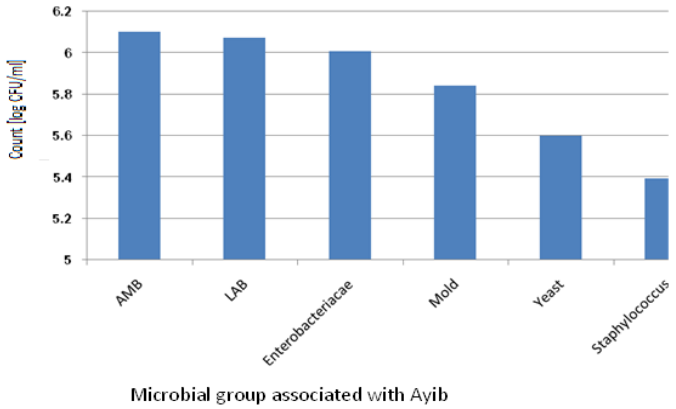

Figure-1 Total count of different microorganisms from street sold Ayib in Jimma town, February 2011. Where: AMB = aerophilic mesophilic bacteria, LAB = lactic acid bacteria.

In this study, the count of LAB was higher as compared to other commonly encountered microbes. This is because LAB can withstand the low pH, since when they grow in acidified medium; they maintain a higher pH in the cytoplasm than the culture medium (Kashket, 1987). This higher internal pH is accomplished by pumping out protons (H^+ ions) via ATPase (Walstra *et al.*, 1999). Similarly, the higher count of yeasts observed in the sample analyzed might be also due to their ability to tolerate low pH and low water activities, grow at low temperature and high salt concentration, to assimilate lactose and organic acids such as succinic, lactic, and citric acid (Fadda *et al*., 2001). Since yeasts are recovered at the end of the fermentation stage, it is suggested that they play a secondary role next to LAB in aroma development (Benkerroum and Tamine, 2004). However, public health significance of yeast in food, including dairy products, has been considered as very minimal (Fleet and Mian, 1987). Rather yeasts are a major cause of spoilage of fermented milk (Vetier *et al*., 2003). Common contaminating yeasts of cheese are *Candida, Kluyveromyces, Marxianus, Geotrichum candidum, Debaryomyces, Hansenii, and Pichia* spp (Johnson, 2001).

In this study, considerable count of molds was observed as the acidic nature of the Ayib is selective for their growth. Even though most molds grow well on the surfaces of cheese when oxygen is present, some heat resistant molds such as *Byssochlamys nivea*, are capable of growing in cheese of reduced oxygen (Taniwaki, 1995).

In general the cheese microbiota, whose community structure evolves through a succession of different microbial groups, plays a central role in cheese-making. Therefore, addition and surface-ripening cultures marketed today for smear cheeses are not sufficient for adequately mimicking the real diversity encountered in cheese microbiota. The interactions between bacteria and fungi within these communities determine their structure and function. The interactions offer to enhance cheese flavor formation and to control and/or prevent the growth of pathogens and spoilage microorganisms in cheese (Irlinger and Mounier, 2009).

Biochemical characteristics of isolates showed that all isolates were motile, rod type of cell shape and catalase positive (Table 3). Most isolates (n= 174) identified from the Ayib samples were Gram positive with few frequency (13.1% (n = 24/198)) of Gram negative. The contributing factor for the low prevalence of Gram negative bacteria in the Ayib sample analyzed might be unfavorable pH which could not support growth of pathogenic bacteria group which can grow in neutral pH. This showed that LAB observed in higher quantity may also have a role in inhibiting the growth of pathogenic and spoilage bacteria by altering the pH (Pacheco and Galindo, 2010). Contaminated cheese has been responsible for outbreaks of food poisoning by several types of bacteria and sporadic cases of illness associated with contaminated cheese have also been reported (Khan and Rockliff, 2002). But due to its acidic property of the media potentially pathogenic microbes cannot survive in a cheese. Thus significant raw milk acid cheese associated outbreaks of human diseases reported are not common (Pacheco and Galindo, 2010).

Table 3 Biochemical test results of isolates from street sold Ayib in Jimma Town.

Media	No of isolates	H_2O_2 (catalase test)	KOH (Gram rxn)	Cytochrome oxidase	Morphology	Cell arrangement	Motility
PCA	4	+	-	-	Rod	Cluster	Motile
PCA	26	+	+	+	Rod	Single	Motile
PCA	45	+	+	-	Rod	Cluster	Motile
PCA	75	+	+	-	Rod	Chain	Motile
MacC	20	+	-	-	Rod	Tetrads	Motile
MacC	15	+	+	+	Rod	Single	Motile
MacC	13	+	+	-	Rod	chain	Motile

NB: PCA = plate count agar, MacC = MacCon

CONCLUSION AND RECOMMENDATION

The Ayib handlers assessed follow a traditional procedure of Ayib processing which is vulnerable to contamination. Possibly their poor hygienic practice and handling can contribute to the higher level of microbial load. As the Ayib samples analyzed were found in acidic range, it is certain that acid tolerant LAB, yeast and molds are found in higher load. In this study, even tough the load of Gram negative bacteria observed was lesser, it could be an indicator for the presence of pathogenic contaminants. Thus, bodies concerned and sanitary workers should give more emphasis to monitoring the hygienic status of Ayib ready for consumers. Moreover, training for Ayib producers and seller on dairy product handling, processing and packaging by local health extension workers is recommended.

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