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Microbiological quality of *Ayib*, traditional Ethiopian cottage cheese, in Jimma area, South-West Ethiopia

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This study was conducted on randomly selected 150 samples of soft cheese (*Ayib)* collected from five vendors of open market places at Jimma town and its surrounding districts: Dedo, Seka, Serbo and Yebu markets to determine microbial quality. Samples were taken three times from the same vendors at different market days with sterile screw cap bottles while categorized into two: *leselasa* (soft) and *derake* (dried) soft cheeses. Samples were collected following standard procedures and then cultured on plates of aerobic plate count (APC) agar, violet red bile lactose agar (VRBL agar) and violet red bile glucose agar (VRBG agar) for aerobic mesophilic bacteria, coliforms and Enterobacteriaceae count, respectively. Bacterial identification was done by culturing on selective medium and biochemical test using Bergey's Manual of Systematic Bacteriology Identification flow chart. The mean count of coliforms, Enterobacteriaceae and aerobic mesophilic bacteria count were 5.709, 6.504 and 8.844 cfu/g, respectively. Significant difference (P<0.05) was observed between places in all microbial count parameters and between categories in coliforms and Enterobacteriaceae count. The dominant bacterial pathogens isolated from *Ayib* samples in decreasing order were *E. coli* (20.67%), *Staphylococcus aureus* (18.00%), *Vibrio* spp. (12.67%) and *Vibrio parahemolyticus* (12.00%); and also *Pseudomonas aeruginosa*, *Salmonella* spp., *Staphylococci* spp., *Shigella flexneri*, and *Proteus mirabilis*. The high bacteria count, and isolates of different species of pathogenic suggested higher risk of public health and hence the need for improved hygienic practice at all levels during milk production and processing.

Key words: *Ayib*, aerobic mesophilic bacteria, coliforms, cottage cheese, Enterobacteriaceae, Jimma.

INTRODUCTION

Cheese is a popular food due to its diversity in application, nutritional value, convenience and good taste (Farky, 2004). *Ayib*, Ethiopian cottage cheese is important in the Ethiopian diets. It may be consumed fresh as a side dish, or it may be spiced salted and mixed with herbs (Almaz et al., 2001).

Ayib is made from the churning of sour milk which results in the removal of fat. Churning of sour milk is carried out, by slowly shaking the contents of the pot until the fat is separated. The fat is then removed and buttermilk is heated in a clay pot or other cooking utensils on a low fire at temperatures varying between 40 and 70°C which results in the formation of a curd (O'Mahoney, 1988; FAO, 1990; O'Connor, 1994). Temperatures higher than

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80°C are reported to give the product cooked flavors (O'Mahoney, 1988). After gradual cooling, the curd is recovered from the whey removing the whey through draining and is ready for consumption fresh or with added ingredients for flavor. *Ayib*, butter and sour milk are usually marketed through the informal market after the households have satisfied their needs (Tsehay, 2001). On the market, sellers and customers categorize into *leselasa*/*erteb(soft)* and *derake (dry) Ayib*.

It is known that cooking of the curd is expected to decrease the microbial load which has an acceptable level at consumption. However, *Ayib* collected from market contained high counts of aerobic mesophilic bacteria (log 8 cfu/ml) (Ashenafi, 1990a; Ashenafi, 1990b; Ashenafi, 1994). The sources of contamination could be from handlers, and utensils, used for packaging and possibly imparting flavor (Ashenafi, 2006). Water used from boreholes wells and rivers to cleaning the udders and milking utensils could also affect the milk quality, mainly at peri-urban farms (Yitaye et al., 2009). Additionally, traditional milk processing and utensils used for storage are often porous and therefore creating a reservoir for many organisms and difficult to clean (O'Connor, 1994).

In spite of this, there is limited information documented on the microbial quality of *Ayib* in the current study area, hence understanding and analysing the situation is crucial for improved interventions on hygiene conditions and practices of the postharvest product quality at both local and national level. Therefore this study was conducted specifically to evaluate the microbioligical quality of *Ayib* and to identify possible isolates that might contaminate it.

MATERIALS AND METHODS

Sampling

The study was conducted in Jimma town, and its surrounding areas: Serbo, Yebu, Seka, and Dedo located within a 25 km radius and which, are the direct suppliers of *Ayib* to the Jimma market. One hundred and fifty samples of *Ayib* were collected from randomly selected five open market vendors, that is, from regular selling vendors No. 21, 24, 19, 28 and 20 found in the market places of Serbo, Yebu, Seka, Dedo and Jimma, respectively, between March and June, 2010. Samples were taken three times from the same vendors every week, stored and kept cold in sterile screw cap bottles and marked: *leselasa* (soft) and *derake* (dried) and transported in ice contained icebox to Jimma University College of Agriculture and Veterinary Medicine's Microbiology Laboratory for analysis.

Microbiological analysis

Preparation of test sample and decimal homogenates

Twenty-five gram of *Ayib* samples were diluted with 275 mL sodium citrate solution at 45°C and pH 7.5 of the diluted solution. Further decimal dilutions were carried out at 1:10 ratios with peptone saline diluents (1g peptone and 8.5 g sodium chloride per 1 L of distilled water) at an ambient temperature.

Aerobic mesophylic bacterial count

0.1 mL of each dilution from 6^{th} to 3^{rd} serial dilution was inoculated onto the centre of pre dried plates of aerobic plate count agar (Difco) and incubated at 30-32°C for 48 ± 2 h. Average counts were calculated, where possible, using dilutions giving 30 to 300 colonies (NSMSOPF, 2005).

Coliforms count

1 mL of each decimal dilution from 6^{th} to 2^{nd} serial dilution was placed on sterilized Petri dish. Molten tempered violet red bile lactose agar was poured into an inoculated Petri dish and incubated, inverted at 30°C for 48 ± 2 h. Characteristic colonies, which were dark red with a diameter of at least 0.5 mm were counted. Average counts calculated from dilutions were from selected plate counts of between 15 and 150 (Richardson, 1985).

Enumeration of Enterobacteriaceae by the colony count technique

1 mL of each decimal dilution from 6^{th} to 2^{nd} was inoculated on sterilized Petri dish. Molten tempered violet red bile glucose agar were poured into each Petri dish; and incubated invertly at 30°C for 24±2 h. Colonies produce purple red with a diameter of 0.5 mm or greater and sometimes surrounded by a red zone of precipitated bile containing 15 to 150 colonies was recorded. Five suspected colonies from the highest dilution were selected and sub-cultured onto a nutrient agar (Oxoid) plates; and incubated at 37±2°C for 24±2 h. Ten (10) to 15 colonies obtained from the higher dilution of plate count agar plate concerned the morphological differences; and confirmed *Enterobacteriaceae* colonies from violet red bile glucose agar plates were identified by using selective medium and biochemical test (NSMSOPF, 2005).

Identification test from aerobic mesophylic bacterial count

Gram positive and negative organisms were categorised by using potassium hydroxide test. Gram negative rod organisms were under taken for oxidase test then, cultured on nutrient agar plate. Gram positive organisms were subcultured on 5% blood agar base (HIMEDIA) and mannitol salt agar (HIMEDIA). Colonies found from blood agar were categorized based on hemolytic activity and sub-cultured on bile esculin agar (CDH) and nutrient agar (for catalase test). Tube coagulase test was under taken for identification of *Staphylococcus aureus, Staphylococcus spp*. and *Micrococcus spp.* Gram negative and oxidase positive organisms were sub-cultured on triple sugar iron agar (Oxoid) to confirm glucose fermentation of *Pseudomonas species.* Thiosulfate citrate bile salts sucrose agar (Difco) was used for the selective isolation of *Vibro cholerae* and *Vibrio parahaemolyticus* and *Aeromonas hydrophila* was isolated with starch ampicillin agar (beef extract 1.0 g, proteose peptone No. 3 10.0 g, sodium chloride 5.0 g, phenol red 0.025 g, agar 15.0 g, soluble starch 10.0 g per 1 L distilled water sterilized and cooled (50°C) the medium with added 0.01 g of ampicillin dissolved in a very small quantity of distilled water).

Identification test from violet red bile glucose agar plates

Confirmed colonies were undertaken for indole, citrate, urease, MR-

Table 1. The mean bacterial counts (log_{10} cfu/g) in the *Ayib* samples of different places and categories.

Place	Coliform	Enterobacteriaceae	Total bacteria
Overall mean	5.709±0.190	6.504±0.191	8.844±0.107
Dedo	4.691 [b]	6.088[a]	8.356 [c]
Jimma	4.950 [b]	5.658[b]	8.632 [ab]
Seka	4.781 [b]	5.710	8.406 [c]
Serbo	4.659 [b]	5.509	8.471 [bc]
Yebu	5.386 [a]	6.222[a]	8.836 [a]
Standard error (±)	0.138	0.138	0.076
Category			
Derake Ayib	4.731[b]	5.691[b]	8.555[a]
Leselasa Ayib	5.056[a]	5.983[a]	8.525[a]
Standard error (±)	0.091	0.090	0.048

*Means in a column with the same letter are not significant different (*P≥0.05*).

VP, lysine decarboxylase, H_2S production and motility tests using sulfide indole motility medium (Oxoid), urea broth (Oxoid), methyl red-voges proskauer broth (HIMEDIA), lysine decarboxylase broth (CDH), Simmons citrate agar (Oxoid), Kligler iron agar (Oxoid), xylose lysine desoxycholate agar (HIMEDIA), MacConkey agar (Oxoid) and triple sugar iron agar.

Statistical analysis

Log_{10} transformed values of the microbial composition quality determination parameters were analyzed using mixed linear models with Statistical Analysis Software (SAS) version 9.1 (SAS Institute Inc. USA, 2008). The least square difference was used to separate means when the parameter tests were significantly different at P< 0.05.

The fixed-effects parameters are associated with known explanatory variables, which were places, categories and interaction effect of place and category. However, the covariance parameters were needed because of the covariance parameters arising in application, which results in additional unknown random effects (vendors) that could affect the variability of the data. Vendors were nested within places, since they were varied between places. Thus, the PROC MIXED was used to analyze both fixed and random effects of the experiment. PROC MIXED was fitted using the default mixed linear model of Restricted Maximum Likelihood (REML) and produced appropriate statistics.

RESULTS AND DISCUSSION

Bacterial counts

The mean coliforms, enterobacteriaceae and total bacterial count were significantly (*P*<0.01) different between places. In addition, there were significant difference in coliforms (*P*<0.01) and Enterobacteriaceae (*P*<0.05) count between categories (Table 1). The coliform count of Yebu, Enterobacteriaceae count of Dedo and Yebu, and total bacterial count of Yebu except from Jimma were significantly higher than from other places of respective bacterial counts (Table 1). In spite of categories, the mean count of coliforms and Enterobacteriaceae from wet (*leselasa) Ayib* was significantly higher than that of the dry *(derak) Ayib*.

According to the Council of the European Communities standard (Council Directive 92/46 EEC 1992), the coliform count from our samples: 26.67% of Dedo, 33.33% of Jimma, 13.33% of Seka, 26.67% of Serbo and 40.00% of Yebu *derake Ayib*s; and 66.67% of Dedo, 53.33% of Jimma and 66.67% of Yebu *leselasa Ayib* had unsatisfactory quality. Soft (*leselasa)* and dry (*derake) Ayib* of Jimma were beyond the satisfactory quality. Higher percentages of *derake Ayib* samples had acceptable microbial quality than from *leselasa Ayib* of all sites except Serbo (Figure 1).

Related literature values show that the count of Enterobacteriaceae in the cheeses made from heat treated milk is 10^3 cfu/g (CEC, 2005), and in the ready-to-eat foods more than 10^4cfu/g is unsatisfactory (Gilbert et al., 2000); whereas the count of all samples, in this study were beyond this points. However, the major proportion of the total samples from both *dereke* and leselasa *Ayib*, were found over the upper limit standard of the cheeses made from raw milk, which is 10^5 cfu/g (CEC, 2005) (Figure 2).

In addition, the maximum limit of aerobic mesophylic bacterial count which is commonly employed to indicate the sanitary quality of food, for raw milk intended for processing is 10^5 cfu/ml and intended for direct human consumption is $5x10^4$ cfu/ml (Council Directive 92/46 EEC 1992; Bodman and Rice, 1996); whereas the count from all samples indicated above this standard.

Nevertheless, this finding is in line with the finding of Zelalem et al. (2007), who found 4.4, 5.1 and 7.9 log cfu/g of coliforms, Enterobacteriaceae and total bacterial count from *Ayib*, respectively; and also Ashenafi (1990a) found 8log cfu/g of total bacterial count in *Ayib* samples collected from an open market.

This higher number of coliforms, Entrobacteriacae and total bacterial count might be due to microbiological quality and composition of milk types and water used for

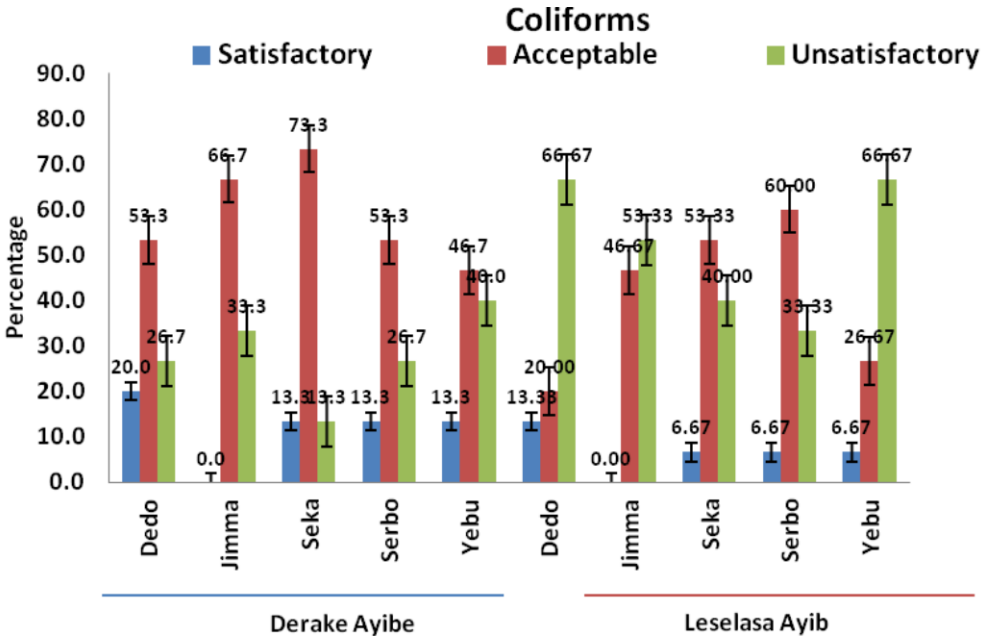

Figure 1. Coliforms count standardization in percentage according to the Council Directive 92/46 EEC, 1992 standard of soft cheese made from heat-treated milk. Satisfactory if the countis ≤10000 cfu/g; unsatisfactory if the count is ≥100000 cfu/g; acceptable between 10000 and 100000 cfu/g.

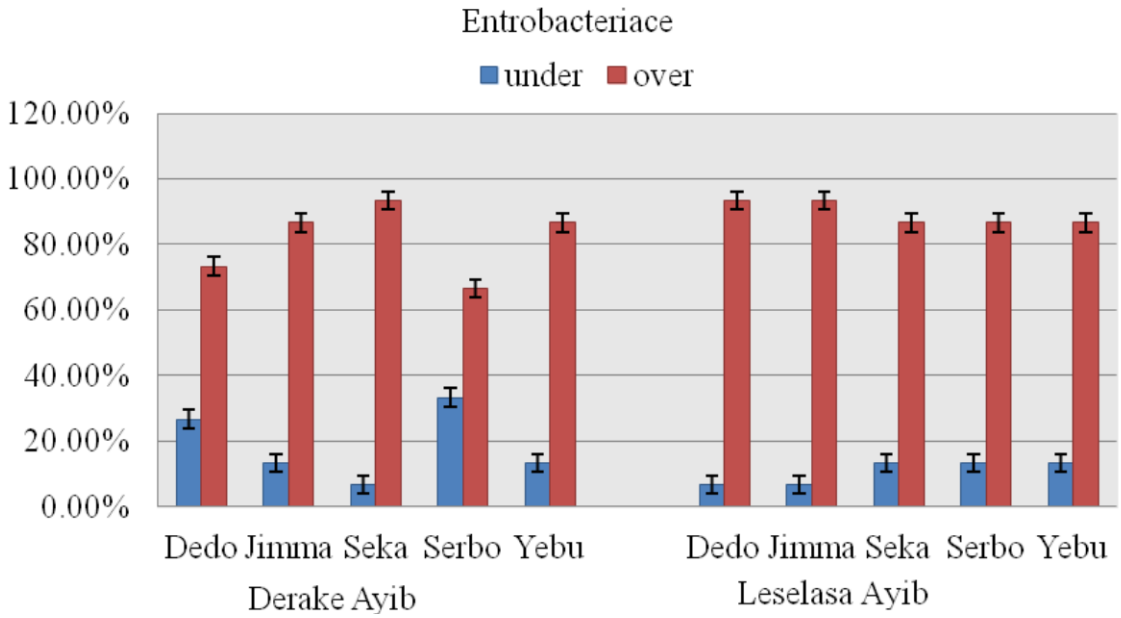

Figure 2. Entrobacteriace count standardization in percentage according to the EU, the value for cheeses made from raw milk standard 10^5cfu/g (CEC, 2005).

cleaning processing utensils (Marth and Steele, 2001). On the other hand, traditional milk utensils are also porous and a reservoir for many organisms and difficult to clean (O'Connor, 1994). In addition, cleanness of the milking and processing utensils (Almaz et al., 2001) and the variation of curd cooking temperature (Ashenafi, 1990b) could contribute to higher bacterial count. Milk handler's activities such as coughing, sneezing, scratching and from body surfaces in contact with milk or milk products, particularly the fingers (Marriott, 1995);

Table 2. Bacterial isolates and number of samples it occurred from the higher decimal dilution in *Ayib* samples.

Isolate	*Derake Ayib* ($i^a ln^b$)					*Leselasa Ayib* (*iln*)					% occurred per N^c
	Dedo	Jimma	Seka	Serbo	Yebu	Dedo	Jimma	Seka	Serbo	Yebu	
Escherichia coli	7		6	5		3		4		6	20.67%
Staphylococcus aureus	3	5		4	2	5	5			3	18.00%
Vibrio spp.	3		1	4		5	3			3	12.67%
Vibrio parahemolyticus		2		2	3	3		1	1	6	12.00%
Pseudomonas aeruginosa		4		6					3		8.67%
Salmonella spp.		5			3		2	2		1	8.67%
Proteus mirabilis		2	1				4		3	1	7.33%
Proteus vulgaris				3					2	4	6.00%
Staphylococci spp.		2		1		3	1			1	5.33%
Enterococcus faecalis					4	3					4.67%
Shigella flexneri			1	1		2			1	1	4.00%
Erwinia cacticida		2							3		3.33%
Providencia stuartii		2			2						2.67%
Aeromonas hydrophila	1		2								2.00%
Streptococcus pneumonia		2									1.33%
Total isolates			91					85			176

[a]Number of samples which the isolates occurred; b, sample size per place (*n*=15); c, total sample size (*N*=150).

and plant parts used for packaging and for imparting flavor which could be contaminated with soil (Ashenafi, 2006) would increase the bacterial count. Besides, coliforms are associated with faecal contamination, and can be killed by pasteurization. They may be found in the soil, on vegetables and in untreated water (Teka, 1997). Thus its existence is considered as indicators of post-pasteurization contamination as a result of poor sanitation (Jayarao et al., 2004).

Bacterial isolates

Total bacterial isolates from the higher dilution of *Ayib* were 176 (91 from *derake Ayib* and 85 from *leselasa Ayib*). From these, the frequently identified organisms were *E. coli* (20.67%) and *S. aureus* (18.00%) with the highest percentages followed by *Vibrio spp* (12.67%), *Pseudomonas aeruginosa* (8.67%), *Salmonella spp.* (8.67%), *Staphylococci spp.* (5.33%) and *Shigella flexneri* (4.00%) in decreasing order (Table 2).

Several Staphylococcal species other than *S. aureus* reportedly produced staphylococcal enterotoxins (Jay, 1992). Their presence could be due to contamination of *Ayib* during and/or after processing. The occurrences of most food toxins caused by *S. aureus* were the result of bad hygienic practices in household and industrial kitchens (Klaske, 2008). Similar isolates of *S. aureus* were obtained from *Ayib* samples collected from open market in Awassa (Ashenafi, 1990a) and from milk and milking utensils in Jimma town (Tadesse and Solomon, 2003).

Enterococcus faecalis, E. coli, Salmonella species and *S. flexneri* were indicators of poor hygienic practices. *E. faecalis* are normal inhabitants of the gut flora of humans (Mitsuoka, 1992) and animals (Willard et al., 2000); therefore, the presence of such organisms indicates contamination with animal or humane excreta. The presence of *E. coli* was reliable indicator of fecal pollution generally in sanitary conditions of water source and milk (Soomro et al., 2002). Similar observation was recorded from milk samples in Jimma town (Tadesse and Solomon, 2003). *Salmonella species* are disseminated in

the natural environment (water, soil, sometimes plants used as food) and through human or animal excretion (Todar, 2008); therefore, its presence in the *Ayib* samples could be derived from water, soil and / or faeces.

Additionally, it might be associated with foods prepared in contaminated kitchens (Dufrenne et al., 2001; Kusumaningrum et al., 2004). The presence of *Shigella* spp*.* in *Ayib* would indicates the contamination with infected food handlers, sewage, water used for cleaning, flies breed in infected faeces and/or soil of infected area; cause they are acquired from such (Todar, 2008).

Vibrio species and *Aeromonas hydrophila*

Vibrio species are widely distributed in aquatic environments and can be readily isolated from water, sediments, and seafood. *A. hydrophila* is also found in the vast aquatic environments (Hazen et al., 1978), fresh water, water supplies, sewage and also in soil (Bremer et al., 2003). Therefore, the presence of these organisms would be derived from water used for production and processing. Some of the *Vibrio species* are pathogenic to humans, like *V. cholerae* and *V. parahaemolyticus* which cause foodborne diseases (Jaksic et al., 2002; Baffone et al., 2005).

Conclusions and recommendations

The high level of counts and isolated pathogenic bacteria in the *Ayib* represent poor product processing and handling and this could result in public health risk to the consumers. This suggests the need for improved hygiene practices at all levels in the dairy value chain. Focused group of governmental and non-governmental organizations should strengthen awareness campaigns on improved hygiene practices so as to reduce the postharvest quality losses and the rate of microbial infections with food poisons due to the possible presence of Enterobacteriaceae and other bacterial pathogens.

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