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Assessment of Microbial Quality and Safety of a Traditional Fermented Milk-'*Irgo*', Collected from Hawassa City, South Ethiopia

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Abstract

Milk in Ethiopia is mainly produced under three livetsock production systems, notably the pastoral, the mixed crop livestock and urban/peri-urban dairy systems. Although proximity to market favors urban dairy producers, there still exist various challenges among which are safety and quality issues. This study was initiated to assess the microbial qualities and safety of fermented milk–*Irgo*, which is one of the most prevalent forms of dairy products produced and marketed by urban dairy producers and intermediate traders in Hawassa city, southern Ethiopia. A total of 120 samples (raw milk=60 and *Irgo*=60) were collected from dairy shops in Hawassa city. Formal interviews on milk and Irgo handling practices were followed by microbial analysis of the products. The mean aerobic mesosphilic bacterial count (AMBC), coliform count (CC), Staphylococcus count (Staph. C) and lactic acid bacterial count (LABC) of the raw milk samples was 6.85, 6.14, 6.13 and 7.19 log cfu ml^{-1}, respectively. *Irgo* samples had mean AMBC, CC, Staph.C and LABC values of 6.79, 5.6, 5.55 and 6.13 log cfu ml^{-1}, respectively. Although, the counts of hazardous microbes were lower in *Irgo* samples than the raw milk, the overall microbial count in the sampled products is much higher than the minimum standards, which reveals the poor handling practices of dairy products in the city. This poor handling of dairy products have consequences to the public health, hence it require due attention in order to minimize its effect on the health and safety of consumers.

Keywords: Milk handling; Microbial quality; *Irgo*; Hawassa

Introduction

Milk is one of the major livestock comodities in Ethiopia, which is produced under three major livetsock production system; the pastoral, the mixed crop livestock and urban/peri-urban dairy systems. Due to its short shelf-life, milk has to be consumed immedietly or processed into shelf stable products. Milk processing is one of the oldest traditional practices in Ethiopia, which is generally based on *Irgo* (a traditional fermented milk product) [1,2]. The fermentation process does not require additions of any defined starter culture, rather natural lactic acid bacteria (LAB) spontaneously ferment the milk. Raw milk is either kept at ambient temperature or kept in a warm place to ferment prior to processing [3]. This *Irgo*, which is analogues to the commercial yoghurt, can be consumed as it is, or can be further processed into other fermented dairy products, notably cottage cheese and butter [4].

The total bacterial count (TBC) of milk samples is indicative of herd health status, farm sanitation, and milk storage temperature operated with. Campylobacter, enterohemorrhagic strains of *Escherichia coli, Salmonella* and *Yersinia* are often implicated in milk-borne diseases [5]. Some of the pathogenic organisms are also found in Ethiopian dairy products including in the fermented product- *Irgo* [3,6]. The fates and development trends of microorganisms of some pathogenic microbes in the fermentation process of *Irgo*, such as *Escherichia coli* 0157:H7, during fermentation process of *Irgo* have been documented [7,8]. The studies indicated the potentials of fermentation process in minimizing the risks of pathogenic organisms in such products. Furthermore, some traditional practices like smoking of milk utensils by fumigation indigenous herbs also minimizes the multiplications of some pathogenic microorganisms [9].

As indicated in the works of Sintayehu et al. [10] about 1,470 urban resident households were engaged in urban dairy farming in Hawassa city, one of the major cities in Ethiopia. An estimated 4,257,110 liters of milk is annually produced. The majority (79.2%) of urban milk producers target milk market, while only 14.2% and 6.6% produced for own consumption as raw milk and home processed products,

respectively. Hotels, coffee houses, *Irgo* sellers and other consumers were the main buyers of raw milk. Milk is usually consumed along with coffee, tea or as it is prior or without boiling. Particularly in Hawassa city, quite large number of *Irgo* selling shops exist, which are owned either by milk producing farms or intermediate traders.

During the manufacturing process of industrial/commercial fermented dairy products, such as yogurt, pasteurization of the milk is a prerequisite process-step before inoculation of the milk with fermentative starter cultures. This process destroys pathogenic and spoilage microflora from the products [11] making the fermented milk safer for consumers. It is unusual to practice pasteurization of milk prior traditional fermentation of milk in Ethiopia, as it may disturb the spontaneous fermentation process. *Irgo* sellers in Hawassa city in particular, usually buy the raw milk in the morning hours, pour it in a small glass/cups (having a capacity of about 200-250 ml) and keep it in an open air (at an ambient temperature) for about 12-24 hours until it naturally gets fermented.

The above mentioned practices in the city, the climatic conditions of the area and the peculiar microbial properties of milk, are suggestive for high risks of microbial quality and safety on the product-*Irgo*. Therefore, the present study was designed to assess the milk handling practices and quantify the microbial quality and safety of raw milk and *Irgo* in the city.

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Materials and Methods

Description of study area

This study was conducted in Hawassa city, capital of the Southern Nations Nationalities and Peoples regional state (SNNPRS). The city is located at an altitude of 1750 m.a.s.l at 6°83' to 7°17' N and 38°24' to 38°72' E, which is 275 km south of the capital Addis Ababa. According to the regional meteorological agency, the data for three decades reveals that this area has annual average rainfall of 955 mm with mean annual temperature of 20°C (SNNPRS-RSA, 2006). The city is the economic and cultural hub of the region, having a total area of about 50 km^2 divided into eight sub-cities (Kifle ketema) and 32 Kebeles (the smallest unit of administration). The total human population of the city during 2008 was estimated at 300,000. About 1,400 urban dairy producers existed during the same year [10].

Diagnostic survey

A formal survey was conducted using open and closed questionnaires. The questioner contained questions on the sources, production process, handling practices, and transportation of *Irgo*. A total of 73 *Irgo* producing shops were sampled from a total of 307 shops in the city. Based on their source of milk for *Irgo* preparations, the *Irgo* shops comprised three groups: group A were those who take milk from own farms (n=6), group B purchase milk from contact farms usually from single source (n=36) and group C purchase milk from various farms/retailers without prior contractual agreements, usually from multiple sources (n=31). The survey was conducted between December 2011 and July 2013.

Sampling of milk and *Irgo*

Following the survey, a total of 120 (60 raw milk and 60 naturally fermented milk *Irgo*) samples were aseptically collected from the interviewed *Irgo* producers/sellers, while 13 farmers refused to give samples. The milk and *Irgo* samples were labeled by the shop names and stratified according to sources of raw milk, group A-C (Table 1).

Laboratory analysis

pH measurement: pH of milk and *Irgo* samples were determined using a digital pH meter (pH meter 704, Metrohm ion analysis, Metrohm ltd., Herisau, Switzerland) after calibrating it using standard buffers of pH 4 and 7.

Microbial enumeration and isolation: Appropriate decimal dilutions of milk and *Irgo* samples were plated with separate sterile pipettes after each plate was labeled with the sample numbers. Before removal of the milk samples from storage containers, the content was mixed thoroughly and vigorously with whirl mixer. Finally, test portions were surface plated on respective culture media following recommended standard laboratory protocols. Specific procedures employed for the enumeration of the groups and specific pathogens considered are briefly described:

Aerobic Mesophilic Bacterial Count (AMBC): Each of one ml

sample were dispensed into sterile test tubes in 0.1% peptone water (Oxoid, UK), as 1:9 portion of the peptone water for initial dilution. Duplicate serial dilutions were made by transferring 1 ml of the previous dilution in 9 ml of 0.1% peptone water. A plate count agar (PCA) (Oxoid, UK) media was used to grow the bacteria at incubation temperature of 32 ± 2°C for 48 hrs. AMBC was made by incubating surface plated duplicate decimal dilutions of milk samples on. After incubations, petridishes with about 30 to 300 per plates were considered for colonies counts [12].

Coliform Count (CC): One ml of milk and *Irgo* samples were dispensed into sterile test tubes containing 9 ml of 0.1% peptone water (Himedia, India) and thoroughly mixed using whirl mixer. Subsequent serial decimal dilutions were prepared in a similar manner using 0.1% peptone water. Duplicate appropriate decimal dilutions were surface plated on Violet Red Bile Agar (VRBA) (Pharma, US) and incubated at 45°C for 24 hours. After complete incubations, typical dark red colonies on uncrowned plates were considered as coliforms for colony counts. This was followed by a confirmatory test by transferring five colonies from each plate to tubes of 2% Brilliant Green Lactose Bile Broth (BGLBB) (Oxoid, UK). Gas production after 24 h of incubation at 32°C was considered sufficient evidence of presence of coliforms [12].

Lactic Acid Bacteria count (LABC): One ml of appropriate serial dilutions in peptone water of raw milk and *Irgo* samples were added into a sterile dish. A molten MRS Agar (Oxoid, UK) (45°C) was then poured onto the dish and mixed thoroughly. After the medium had set, another layer of MRS Agar was poured over the surface to produce a layer-plate. Colonies were counted after plates were incubated at 35°C in an atmosphere of 5% CO_2 for 48 hours [13].

Total Staphylococci Counts *(Staph.C)*: milk and *Irgo* samples were thoroughly mixed and plated on mannitol salt agar (MSA) plates. The dried plates were incubated for 45 to 48 hr at 35°C. Typical *Staphylococci* colonies appeared as golden yellow, smooth, circular, convex, and moist were counted. For confirmation, four to five of typical colonies per MSA plate were streaked on Mannitol salt agar (Oxoid, UK), which was followed by catalase test and Gram stain [14,15].

Data analysis

Descriptive statistics were employed to analyze data collected from *Irgo* shops having different sources of milk for *Irgo* processing. Qualitative data were analyzed using descriptive statistics. The results of microbial counts were first transformed to logarithmic values (log 10) and these transformed values were analyzed using the General Linear Model (GLM) for least squares means in SPSS (version 16) using a fixed effect model. The Least Significant Difference (LSD) test was used to separate the means and differences were considered significant at P<0.05.

The numbers of colony forming unit microorganisms were calculated using the following mathematical formula as recommended by [16].

CFU/ml=C/(1*n1 + 0.1*n2) d

Where;

ΣC=sum of all colonies on all plates counted, n1=number of plates in first dilution counted, n2=number of plates in second dilution counted, d=dilution factor of the lowest dilution used.

Results

Characteristics of *Irgo* producers

Among the interviewed *Irgo* producers 82.2% were male and the

Samples	Source of raw milk			Total
	Group A	**Group B**	**Group C**	
Raw milk	6	27	27	60
Irgo	6	27	27	60
Total	**12**	**54**	**54**	**120**

Table 1: Sampling layout used for milk and naturally fermented milk *Irgo* from *Irgo* shops in Hawassa City.

remaining were female (17.8%). The proportion of male and female owners was not comparable in all groups of *Irgo* houses (Table 2). The average age of the *Irgo* shop owners was 24 years and it is comparable for all groups of producers. The overall figure shows that the majority of *Irgo* sellers (63%) practice it at very small scale level utilizing less than 5 liters per day, while followed by 21.9% of them process a milk amount between 6-10 liters per day. Only 15% of them processed a milk of over 10 liters per day into *Irgo*.

Handling of dairy equipments

Irgo producers used different milk containers for storing and fermentations of *Irgo*. About 46.6% of the *Irgo* shops used plastic containers for transportation, processing and storage of raw milk while 43% of the shops used metal containers. In both groups, fermentation of *Irgo* is undertaken in smaller glasses (a 250 ml size). The fermentation duration for the *Irgo* production was reported to be between 10 and 15 hours (68.5%), while others reported about 16 to 20 hours. Limited number of *Irgo* sellers also reported that the fermentation duration was less than 10 hours (1.4%) and more than 20 hours (8.2%).

The survey results showed that cleaning of dairy equipments using warm water and detergents is a common practice by most of the interviewees (90.4%). After complete fermentation of *Irgo* in an open air for about 10-15 hrs, most producers (78.1%) used refrigerator until it is consumed or sold, while 21.9% of did not use any.

Marketing of *Irgo*

The most marketable dairy product in the study area was raw milk, which is followed by *Irgo*, which is prevalently consumed during morning hours along with other breakfast food, notably bread. The selling outlets for the major dairy products were direct to consumers (97.3%) and followed by the retailers (2.7%). Selling to the retailers was observed commonly in the owners who have their own source of milk.

Major problems of *Irgo* shops

As prioritized by the respondent there was Loss of products due to its poor quality (28.8%) followed by challenges related with processing (26%) and selling (15.1%) (Table 3). The reported problem related processing in most of the *Irgo* shops were delay in fermentation, excessive whey production, quality of the raw milk supplied limited milk supply and lack of appropriate equipment. Similarly, fasting season was mentioned in most case as challenge for the marketing of the major products in the shops.

Microbial quality of raw milk and *Irgo* samples

Acidity (pH) level of the milk and *Irgo*: The average pH value of

	Interviewed house *Irgo* producers			Overall (N=73)
Problems	Group A (n=6)	Group B (n=36)	Group C (n=31)	
Loss of products due to its poor quality				
Yes (%)	-	23.3	5.5	28.8
Challenges in selling				
Yes (%)	2.7	4.1	8.2	15.1
Challenges in processing				
Yes (%)	-	19.2	6.8	26.0

Table 3: Potential challenges of *Irgo* shop owners, in Hawassa city.

Type of microbes	Mean microbial count (log CFU ml⁻¹) by sources of raw milk			Mean (N=60)	SD
	Group A (n=6)	Group B (n=27)	Group C (n=27)		
AMBC	4.93 (0.65)[b]	6.8 (0.31)[a]	7.33 (0.31)[a]	6.85	1.60**
Coliform	5.39 (0.43)[b]	6.33 (0.20)[a]	6.13 (0.20)[a]	6.14	1.29**
Staph.C	5.51 (0.41)	6.20 (0.19)	6.19 (0.19)	6.13	1.04[ns]
LABC	6.60 (0.45)	7.18 (0.21)	7.34 (0.21)	7.19	0.95[ns]

AMBC=aerobic mesophilic bacteria count, CC=coliform count, Stap. C=Staphylococci counts, LAB=lactic acid bacteria, SD=standard deviation, NS=non significant ,**significant at $p<0.05$

Table 4: Mean (± SD) microbial counts of raw milk samples (Log_{10} cfu ml⁻¹) collected from three *Irgo* producing groups in Hawassa city.

Type of microbes	Mean microbial count (log CFU ml⁻¹) by sources of raw milk			Mean (N=60)	SD
	Group A (n=6)	Group B (n=27)	Group C (n=27)		
AMBC	5.77 (0.65)[b]	6.82 (0.31)[a]	6.97 (0.31)[a]	6.79	1.72**
Coliform	5.60 (0.43)	5.55 (0.20)	5.64 (0.20)	5.60	0.77[ns]
Staph.C	5.91 (0.41)	5.49 (0.19)	5.53 (0.19)	5.55	0.94[ns]
LABC	6.27 (0.45)	6.21 (0.21)	6.01 (0.21)	6.13	1.20[ns]

AMBC=aerobic mesophilic bacteria count, CC=coliform count, Stap. C=Staphylococci counts, LAB=lactic acid bacteria, SD=standard deviation, NS=non significant,** significant at $p<0.05$

Table 5: Mean (± SD) microbial counts of Irgo samples ($Log10$ cfu ml-1) collected from three Irgo producing groups in Hawassa city.

raw milk and *Irgo* samples collected from *Irgo* shops in the present study were 6.49 and 4.18, respectively. The least pH value observed in the milk sample was 6.44, while in *Irgo* it was 6.07.

Microbial counts the raw milk samples: The microbial quality of raw milk, which is used to prepare *Irgo* is shown as Table 4. The mean AMBC as well as CC of the raw milk samples in group A was significantly ($p<0.05$) lower than group B and C, the later groups having milk source from other farms. This difference however, was not significant for Staphylococcus count and LABC ($P>0.05$). Overall, the highest AMB, Coliform, Staphylococcus and LAB count was recorded for raw milk samples collected from group C, who collects milk from multiple farms and retailers.

Microbial counts in *Irgo* samples: The microbial count of the sampled *Irgo* products is shown in Table 5. The mean AMBC of the *Irgo* samples in group A was significantly ($p<0.05$) lower than group B and C. This difference however, was not significant for Colifom, Staphylococcus count and LABC ($P>0.05$) between the three groups. Overall, the highest AMB, Coliform, Staphylococcus and LAB count was recorded for raw milk samples collected from group C, who collects milk from multiple farms and retailers. The similarities between sampled households for some of the microbial counts, show their closeness in the handling practices of milk and milk products.

	Interviewed house *Irgo* producers			Overall (N=73)
	Group A (n=6)	Group B (n=36)	Group C (n=31)	
Gender (%)				
Male	33.3	88.9	83.9	82.2
Female	66.7	11.1	16.1	17.8
Mean age (± SE yrs.)	24.3 (1.4)	24.0 (1.1)	23.7 (0. 8)	23.9 (0.7)
Amount of milk used for *Irgo* production (%.)				
Less than 5 L	-	94.4	38.7	63.0
6 up to 10 L	16.7	5.6	49.1	21.9
More than 10 L	50.0	-	12.9	9.6
More than 20 L	33.3 (2)	-	6.5 (2)	5.5 (4)

Table 2: Characteristics of *Irgo* producers, in Hawassa City.

Discussion

The present study has highlighted the handing practices and microbial qualities of *Irgo* before and after fermentations. Although milk is fermented in an open air for quite long time, which is good enough to allow various microbes to grow, quite high number of *Irgo* producers used refrigerator after the *Irgo* ferments. The handling of milk and *Irgo* during transportation, storage and processing were generally poor. This was common particularly for *Irgo* shops who take milk from multiple farms and those who do not follow strict sanitary practices. According to Van Kessel et al. [17], the use of insufficient and poor quality water for cleaning of milk handling equipments can result in milk residues on equipment surfaces that provide nutrients for the growth and multiplication of bacteria that can then contaminate the milk.

The Acidity in dairy products at any time is a rough indication of the age of the milk and the manner in which it has been handled [12,18]. The average pH value of raw milk and *Irgo* was 6.49 and 4.18, respectively. Under normal circumstances fresh milk should have between 6.6 to and 6.8 pH at $20^{\circ}C$ [19,20] The current pH value is comparable with results reported by Rahel [21] for raw milk samples collected from Wolayita Zone.

It is recommended that AMBC count in milk should not surpass 5 $\log_{10}$ cfu ml^{-1} in order for the milk to remain in a reasonably acceptable quality for consumption [22] The overall result of AMBC obtained for milk in this study were high (6.85 log cfu mL^{-1}). Subsequently the milk becomes unsatisfactory for consumption. In the current study, The AMBC for milk samples collected from *Irgo* shops was lower as compared to the values reported by Farhan and Salik [23] (8.62 $\log_{10}$ cfu ml^{-1}), Bekele and Bayleyegn [24], where the count reached 8.0 $\log_{10}$ cfu l up on arrival at the processing plant in Addis Ababa, Ethiopia. It was higher than the count value reported by Abd Elrahman [25] for raw milk samples (6.63 $\log_{10}$ cfu ml^{-1}). The higher AMBC obtained in the current study could be related to the overall sanitary conditions followed by most of the farms as well as *Irgo* processers. This could be due to the contribution of insufficient preparation of the udder, insufficient cleaning of milk handling equipments in the farm and *Irgo* shops, use of poor quality water for cleaning, the storage time starting from the production site to the selling points. Murphy and Boor (2000), noted that ineffective use of cleaning water without heat treatment and absence of sanitizers tend to fasten growth of less heat resistant organisms.

The presence of CC in milk indicates that the milk has been contaminated with fecal materials and it is an indicator of the sanitary conditions in the production and handling of the milk starting from the production site to the consumer table. The other potential source of contamination can be associated with the use of loosely lose caped storage containers and poor waste disposal methods followed by the dairy farms in the study area (O'Connor, 1994; Farhan and Salik, 2007). The CC value (6.14 log cfu mL^{-1}) for milk samples collected from the *Irgo* shops was higher than the results obtained by Bekele and Bayleyegn [24] (4.11 to 4.85 $\log_{10}$ cfu ml^{-1}), Alganesh et al. for whole milk samples (4.46 $\log_{10}$ cfu ml^{-1}) collected from storage in East Wallega Ethiopia, Nanu et al. for raw milk samples (3.2 log cfu ml^{-1}) at the production point. Also lower result was observed for raw milk samples collected from storage containers at farm level than the reports of Rai and Dawvedi (1990) from India (5.87 $\log_{10}$ cfu ml^{-1}) but comparable with the value reported by Mogessie and Fekadu [9] (5.0 $\log_{10}$ cfu ml^{-1}) for milk samples obtained from collecting utensils and Zelalem and Faye [6] cow's milk samples (6.57 $\log_{10}$ cfu ml^{-1}) collected from different producers in central highlands of Ethiopia. The present value of CC of milk collected from *Irgo* shops is also higher than the values reported by Abd Elrahman et al. [25-28] (5.61 $\log_{10}$ cfu ml^{-1}) from Sudan. A value of 1.5×10^2 cfu ml^{-1}, is the recommended coliform counts in milk which is internationally acceptable [22]. Apart from safety and public health concerns, high contaminations by coliforms results in off flavors in milk and reduce shelf life of dairy products.

Another important pathogen in milk and related products is a Staphylococcus. It is naturally present in milk and often associated with milk born disease due to the ability of some strains to produce heat stable toxins [29]. *Staphylococcus spp.* is among pathogenic microbes that cause minor skin infections and life threatening diseases. Dairy cows with mastitis may be the source of enterotoxigenic *staphylococcus* in raw milk, which may subsequently be commingled with other milk while collecting from cows. In the present study *Staphylococcus C* was lower than what is reported by Tollossa et al. [30] and was higher than the value reported by Haile et al. [31].

LABC (7.19 log cfu mL^{-1}) comprises a major part of the microorganisms. The overall result shows that relatively the count is higher than the count reported by Eyasu and Fekadu. The mean LAB count obtained for raw milk in this study is lower than that reported for raw milk collected from central Ethiopia. This high value may lead to undesirable fermentative acidification of raw milk. Therefore, effective measures should be taken to avoid such kind of over fermentation as well as spoilage.

Slight reduction in AMBC, CC and *Staphylococcus C* was observed in the *Irgo* sampled from the some shops. The reason for the low count could be the high acidity in the final product. The AMBC result of the present study was lower than earlier reports for fermented milk [32,33]. The CC in the present study was higher than other studies in Ethiopia.

Conclusion

The sanitary practices followed at *Irgo* producers during handling, storage and processing were generally poor. The poor quality raw milk, unclean and insufficient cleaning of milk equipments were among the most important sources of milk contamination. The milk is generally exposed to different contaminants when it transferred from one container to another, transported to consumers as well as retailers from the production site without cooling facilities, and with no proper milk containers. Appropriate hygienic practices should be implemented alongside all actors of the dairy value chain. The fast growing dairy enterprise in Hawassa city should be supported in such a way it enhances the income of smallholder dairy producers as well as other value chain actors, however, the safety of consumers should not be neglected.

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