



PHYSICO CHEMICAL AND MICROBIAL QUALITY OF FERMENTED MILK (ERGO) FROM DAIRY COW IN ABRAHAMO AND URA DISTRICTS OF ASOSSA ZONE, BENISHANGUL GUMUZ REGIONAL STATE, ETHIOPIA

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ABSTRACT: Across-sectional systematic random survey method was conducted from September to March, 2023 with the objectives to assess on physical and microbial quality analysis, which was used to investigate and identify microbial quality of fermented milk and to delineate the antimicrobial sensitivity test in Abrahamo and Ura districts. A total of 100 ergo samples were systematically collected and processed for the presence of microbial load, following the standard techniques and procedures. 3.80 and 3.82 log₁₀CFU/ml of urban and rural of mean bacterial counts were recorded in Abrahamo and Ura districts respectively. The mean *Salmonella*, *Staphylococci*, *E.coli*, *Yeast and Mould* counts of urban and rural were 3.31, 3.37, 3.28, 2.03, 1.85 (urban) and 3.39, 3.34, 3.26, 1.95, 1.73 (rural) log₁₀CFU/ml of microbial load was identified respectively. The mean value of fat, protein, lactose and solids-not-fat, salt, density, water contents were 4.81±3.57, 2.63±0.97, 3.78±1.32, 6.35±2.34, 0.53±0.207, 22.68±9.74 and 17.32±26.77 respectively. The minimum and maximum value of pH in ergo samples were recorded as 3.85 and 6.43 respectively. Therefore, the use of standardized procedures in milking and handling of ergo, provision of training on best practice of milk hygiene and handling of ergo for handlers and raising the level of awareness should be noted in the study areas.

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Key words: *Abrahamo, Ergo, Salmonella, E.coli, Staphylococcus and Molds, yeast, and Ura.*

1. INTRODUCTION

Cow milk is among the most important nutritional components in human diet. It is naturally a rich source of protein (3.2-3.4g/100g), fat (3.1-3.3g/100g), energy (247-274 kJ), water (87.3-88.1g/100gm), lactose (4.5-5.1g/100gm) and valuable vitamins (Vitamin A (µg RE): 61; E (mg): 0.08) and minerals like calcium (91-120mg/100g), potassium (132-155mg/100g), phosphorus (84-95mg/100g) and others (FAO, 2013). On the other hand, milk and milk products are an ideal media for the growth and proliferation of an array of microorganisms of various taxonomic origin with some of them being beneficial and others undesirable and also pathogenic (Fernandese, 2009; Tegegn *et al.*, 2020).

Microbiology of milk is among the principal indicators of its quality. Milk drawn from a healthy mammary gland may be contaminated by a number of microorganisms entering the udder through the teat duct. Gram-positive cocci, streptococci, staphylococci and micrococci, lactic acid bacteria (LAB), *Pseudomonas*

spp., *coryne bacteria* and yeast are most frequently found in milk drawn aseptically from the udder (Fernandese, 2009). In cases of infection of the udder (mastitis), large number of microorganisms can be shed into milk. In general, microbial contamination of raw milk can emanate from a variety of sources, *viz*, air, cleanliness of milking equipment, feed, soil, faeces and grass (Coorevits *et al.*, 2008). Previous surveillance systems had reported that 2-6% of bacterial food-borne outbreaks are shared from milk and milk products. Some wild type molds, such as, *Aspergillus* are capable of producing mycotoxins (Aflatoxin B1, M1, G1) that are secondary metabolites with carcinogenic, teratogenic and mutagenic effects (Tegegn, and Soboka, 2020).

Milk production obtained from the subsistence farmers of Ethiopia contributes to about 98% of the annual milk production of the country. Moreover, dairy processing is mostly restricted to the smallholder level which experience low hygienic quality (Alganesh *et al.*, 2009). Weldearegay *et al.* (2012) in their study of hygienic practices and microbiological quality of milk produced

under different farm sizes in Hawassa city reported maximum values of 10.28, 6.52, and 7.13 log₁₀ CFU of aerobic mesophilic bacteria, coliforms and yeasts and molds, respectively per gram of milk sample. Similarly, milk and milk products quality assessment in terms of microbiology from Debre zeit, Adama and Jimma areas reported that mean total bacterial count ranged from 8.3 to 10 Log CFU/mL and the mean coliform count could reach 4 Log CFU/mL (Zelalem, 2009).

Ergo is a traditional, a spontaneously, fermented milk product which has some resemblance to yoghurt. It is thick, smooth and of uniform appearance and usually has a white milk color when prepared carefully. The product is semi-solid and has a pleasant odor and taste. It constitutes a primary sour milk product from which other products may be processed. Depending on the temperature, it can be stored for 15–20 days (Muluken *et al.*, 2016).

Fermentation is a self-limiting process due to the accumulating acids and/or alcohols eventually kill even the fermenting microorganisms themselves. *Ergo* made at homes by putting the milk in smoked vessels and stored for 2–4 days to ferment, at an ambient temperature of 16–180 °C, milk depending on the ambient temperature. The relatively low pH of *Ergo*, ranging from 4.3 to 4.5, enables its further storage (Ali, 2011).

Physical characteristic of raw milk samples collected at Jimma dairy farms and tested, the majority (75%) had yellow white, while 10% had white color. Among the milk samples, the highest percentage (75%) had normal flavor, while 10% of the sample had off-flavor indicating three farms fed their lactating cows with “atella”-liquid by-product of local beer breweries before milking. In addition, adulteration of milk is a common practice in some of the dairy farms elsewhere in Ethiopia. The mean specific gravity of raw milk samples collected from urban dairy farms in this study was 1.027 ± 0.00, ranging from 1.023 to 1.030. The result of the present study was slightly lower than the values of specific gravity of 1.030 reported by Alganesh *et al.* (2007) in eastern Wollega, Ethiopia.

Therefore, the objectives of the research was:

- To assess physicochemical properties in ergo samples,
- To determine the Microbial quality of fermented ergo from raw cow milk in urban and rural area of Abrahamo and Ura Districts.

2. MATERIALS AND METHODS

2.1. Description of the study area

The study was conducted in Abrahamo and Ura districts of Assosa zone from September to march 2023. Asossa district was splitted to Abrahamo and Ura districts. The town is the capital city of Benishangul Gumuz regional state which is 661 km far from Addis Ababa, the capital city of Ethiopia, (CSA, 2018). Asossa zone has 214 peasant association, stretching over an area of 18,340.55 kilometer square, with human population of 270,980. The Benishangul - Gumuz region is located between latitude of 80 30'' and 400 21'' N and longitude of 340 21'' and 390 1'' E and its altitude range is 700-1560 meter above sea level. Annual rain fall is between 900-1500 mm with uni modal type of rain fall that occurs between April and October. Annual temperature ranges between 25- 35^oc. The livelihood of the society largely depends on mixed livestock and crop production having livestock population of 77,688 Cattle, 167281 Goat, 9651 Sheep, 27638 Equines, 279098 Poultry and 66019 beehives (CSA, 2015)

2.2. Research Design

The research design was cross sectional, which was used to carried out physico chemical properties and microbiological quality analysis in the study areas from September to March 2023.

2.3. Target population

Target population was small holder dairy producers, who have Dairy cows, and collectors/ transporters, and vendors from local shops practicing ergo production and marketing in rural and urban areas of Abrahamo and Ura districts. The targeted milk product under investigation is traditionally fermented ergo from cow milk.

2.4. Sampling frame, Sampling techniques and Sample size

A representative household sample for *ergo* sample collection, isolation and enumeration of physicochemical and microbial quality was done from total population number of 135. Then purposive sampling techniques was used. The sample size who attend on dairy cattle production activities in the Abrahamo and Ura district was determined by using (Yamane, 1973) formula with a 95% confidence level.

$$n = \frac{N}{1+N(e)^2}$$

Where :

n= sample size required

N = number of people in the population

e = allowable error (5%)

n= $\frac{135}{1+ 135 (0.05)^2} = 100$

So, the sample size was 100 in order to analyze physico chemical properties and microbial quality of the ergo samples in the Abraham

and Ura districts of urban and rural locations across production channels.

2.5. Study Methods

2.5.1. Ergo Sample Collection and Handling procedures

A 10-15 ml of ergo samples was collected starting early in the morning from milk vending shops and cafeterias, dairy farms and households (farmers) using sterile bottles. The samples were properly labeled, kept in icebox and transported to the Asossa, Regional microbiology laboratory for microbial Analysis.

The samples (approximately 200 ml each) were collected aseptically and immediately placed in sterilized screw capped sampling bottles and put into an ice box (1-5°C) to ensure the suppression of the growth and proliferation of microorganisms (ET ISO 707, 2012) and immediately transported to laboratory for analysis. Upon arrival at the laboratory, sample was put in a (+4°C) refrigerator until they are further processed. Before processing, the samples were tanned at room temperature.

2.5.2. Physico-chemical Analysis of Ergo Samples

The pH values and the contents of treatable acidity (TA), protein, total soluble solids (TSS), solids nonfat (SNF), moisture and fat, were analyzed according to the procedures outlined by (AOAC, 2000). Some physicochemical parameters such as fat, protein, lactose, solids-not-fat (SNF), added water, salts and density were analyzed using Lacto scan (ultrasonic milk analyzer, Milkotronic LTD).

2.5.3. Media Preparation for Microbial Quality Analysis

Standard plate count test is a test which is useful in assessing the number of total viable bacteria in the ergo milk sample based on which the ergo sample can be graded into different categories according to bacterial content in the naturally fermented milk. Tenfold serial dilution up to 10⁶ was prepared for each sample using 9 ml of 0.85% sterile saline water. Pour plate method was used to prepare viable count by adding 1 ml of diluted sample into a petri dish then adding 15-20 ml of sterilized molten standard plate count agar into the petri dish.

The total aerobic mesophilic bacterial counts (TAMBC) and total coliform count (TCC) were determined using sterile standard plate count agar and violet red bile agar (VRBA), respectively. Yeast count (YC) and mold count (MC) were done using sterile Sabouraud Dextrose Agar (PDA) and its pH was adjusted to 3.5 by adding 10 mL of sterile 10% lactic acid to a 1 L volume of the medium. All media except VRBA were sterilized by autoclaving at 121°C for 15 minutes, while VRBA was

sterilized by boiling for two minutes. After sterilization, all media were cooled to 45-47°C in a water bath before use. The preparation of media was generally done according to the instructions given by the respective manufacturers. Peptone water that was autoclaved at 121°C for 15 minutes and cooled to 30°C was used for serial dilution of the ergo samples to determine TAMBC, TCC, YC and MC individually.

2.5.3.1. Determination of total aerobic mesophilic bacterial count (tambc)

TAMBC was determined using standard plate count agar. One mL of raw milk sample was added into a sterile test tube containing 9 ml of sterile peptone water. After thoroughly mixing, the suspension was serially diluted up to 10⁻⁶ and samples from the appropriate dilution (1 ml) were pour-plated using a 15-20 ml of cooled but still molten standard plate count agar (Oxoid, UK) solution and mixed thoroughly. The resulting plates were allowed to solidify and then incubated at 37 °C for 48 hours (Quinn *et al.*, 2002). The plates with colonies ranging from 30-300 colony forming units (cfu) ml⁻¹ were selected for determination of TAMBC (Kiiyukia, 2003). TAMBC was determined as the total number of CFU per milliliter of ergo sample which was calculated using the formula provided by IDF (2004).

$$N = \frac{\sum c}{[(1 \times n_1) + (0.1 \times n_2)]d}$$

Where: N is the number of CFU per milliliter of milk sample; $\sum c$ is the sum of colonies on all plates counted; n₁ is the number of plates in the first dilution counted; n₂ is the number of plates in the second dilution counted; and d is the dilution from which the first counts will be obtained.

2.5.3.2. Determination of total coliform count (CC)

TCC was determined using sterile violet red bile agar (VRBA). One mL of raw milk or ergo sample was added into a sterile test tube containing 9 ml of sterile peptone water. After thoroughly mixing, the suspension was serially diluted up to 10⁻⁶ and duplicate samples (1 ml) were pour-plated using a sterile 15-20 ml VRBA. After thoroughly mixing, the resulting plates were allowed to solidify and then incubated at 32°C for 24 hours (Quinn *et al.*, 2002). After incubation, typical dark red or purplish-red colonies appearing on the plates were counted as coliforms. For confirmatory test, five to ten typical colonies from each plate were transferred into tubes containing 2% Brilliant Green Lactose Bile Broth and incubated at 37°C for 48 hours. Growth and gas production within the incubation period were considered as sufficient evidence for the presence of coliforms.

(Nyalewa, H.E Nonga, 2018). Plates with 15 to 150 cfu mL⁻¹ was used (Kiiyukia, 2003) for determining total coliform counts using the formula provided by IDF (2004).

2.5.3.3. Determination of Yeast and Mold Counts

Yeast count (YC) and mold count (MC) was determined using sterile Sabourd Dextrose Agar (SDA). One ml of ergo sample was added into a sterile test tube containing 9 ml of sterile peptone water. After thoroughly mixing, the suspension was serially diluted up to 10⁻⁶ and duplicate samples of 0.1 ml was spread-plated on pre-dried surfaces of media containing SDA (Oxoid, UK). The plates was then incubated at room temperature 25°C for 3 - 5 days (Roberts and Greenwood, 2003). Creamy to white/gray colonies was counted as yeasts whereas; filamentous (fuzzy) colonies of various colors (yellow, green, light brown) was counted as molds (Yousef and Carlstrom, 2003). When difficulties was faced to differentiate some colonies whether they are yeast or mold, a microscopic examination using the oil immersion objective was carried out to identify whether the cells in the colonies was unicellular or multi-cellular. Plates with 10 – 150 colonies was used for determining yeast and mold counts (IDF, 2004) using the formula provided by IDF (2004); (Bird. *et al.*, J AOAC Int. 2015).

2.5.3.4. Determination of E. coli

Ergo samples (25 ml) was diluted in buffered peptone saline water (225 ml); serial dilution of 10⁻¹, 10⁻², and 10⁻³ was applied in order to quantify this microbial group. Most probable number (MPN) method was used after serial dilutions to estimate the populations of *E.coli*. *E. coli* was enumerated by growing each serial dilution in 3 test tubes in *Escherichia coli* broth (EC-broth) after 24/48 hours of incubation at 45°C (Asmahan *et al.*, 2011).

2.5.3.5. Staphylococcus count

Sterile pipettes was used to place 0.1ml aliquots from each dilution in to two properly labeled mannitol salt agar (MSA) plates (Oxoid, England). The plates was spread and incubated at 37°C for 48hrs. The typical staphylococcus colonies appeared as golden yellow, smooth, circular, convex and moist was counted. For confirmation four to five of typical colonies per MSA plate was streaked on mannitol salt agar, which is followed by catalase and gram staining tests (Weldaragay *et al.*, 2012)

2.6 Data Processing and Analysis

The data on the major variables were stored in Microsoft excel spread sheets to create a database. After checking data for accuracy, the coded data was analyzed employing descriptive statistics and using STATA version 13 (Statistical software for data science version 13). In all analysis, associations were considered to be significant when p<0.05. The data on microbial counts, which was expressed as colony forming unit (CFU) per ml, of each district was transformed into logarithmic scales (log₁₀cfu ml⁻¹) and analyzed using the General Linear Model (GLM) procedure. Variability among the data was separated using Duncan Multiple Range Test (DMRT) at 0.05 α level of probability (in the case of district wise comparison). Means, maximum and minimum values was calculated in descriptive statistics.

3. Result and Discussion

3.1 Microbial count in ergo samples

The present study evaluated the microbiological quality of raw ergo milk in Abrahamo and Ura districts of urban and rural sites. The microbiological condition of safety and hygiene were then assayed using the methods recommended by International Commission on Microbiological Specifications for Foods. The rate of milk contamination particularly increases during milking point, at milk handling equipment of producers, vendors, and consumers, and udder handling point. Aerobic mesophilic bacteria, *Coliform*, *Staphylococcus*, *Salmonella*, *E. coli*, and yeast and mold were one of major micro- organisms which contaminates milking points (Quinn *et al.*, 2002).

3.2 Rural and Urban counts

The overall mean Bacterial count and Fungal (yeast and mold) count of ergo samples were 6.313 x10⁶ cfu/ml in urban, 6.534 x10⁶ in rural. The Fungal count was 1.78x10⁴cfu/ml in urban and 1.45 x 10⁴ in rural areas, which were significantly associated with locations (p<0.05) (Table 1). Statistical analysis results of the aerobic mesophilic bacteria count demonstrated a significant interaction between locations (p<.05) (Table 1). Although the average values are higher than the established limits of the Ethiopian standard authority, significantly (p<0.05) higher average TAMBC for ergo milk samples was obtained in rural areas (3.82 log cfu/ml) than in urban areas (3.80 log₁₀ cfu/ml).

The average values of *E.coli* count was 3.57 log₁₀cfu/ml as presented in Table 1; and significant (p<0.05) variation was observed in the mean values of TCC between the two locations, that was 3.28 log₁₀cfu/ml in urban while 3.26 log₁₀cfu/ml was reported in rural areas. Average *staphylococcus* found in this study was 3.66log₁₀ cfu/ml; however, equivalent average number

of staphylococcus was investigated in (3.37logcfu/ml) urban location than in rural (3.35 log cfu/ml) locations as Table 1 indicated. Similarly, average salmonella count was 3.65 log₁₀cfu/ml, which was 3.31 log₁₀ cfu/ml count was reported in urban and 3.39 log₁₀cfu/ml count was reported in rural so it was associated (p<0.05). Likewise most of the other parameters, significant variations (p<0.05) in fungal counts were found between urban (2.25 log₁₀cfu/ml) and rural (2.16log₁₀cfu/ml) locations. Consistently, it is recommended that AMBC count in milk should not surpass 5 log₁₀ cfu ml⁻¹ in order for the ergo milk to remain in a reasonably acceptable quality for consumption (Michael W. *et al.*, 1992).

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 *ergo* shops was inconsistent as compared to the values reported by Farhan and Salik (Farhan M, Salik S., 2007) (8.62 log₁₀ cfu ml⁻¹), Bekele and Bayleyegn (Godeay B1 and Molia B. (2000), where the count reached 8.0 log₁₀cfu l up on arrival at the processing plant in Addis Ababa, Ethiopia. It was higher than the count value reported by Abd Elrahman (Samia MA *et al.*, 2009) for raw milk samples (6.63 log₁₀ 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 *ergo* processors. This could be due to the contribution of insufficient preparation of the udder, insufficient cleaning of milk handling equipment's in the farm and *ergo* 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.

Comparably, Yigrem S, Welearegay H. (2015) indicated that, AMBC, CC, Staph and LABC values of 6.79, 5.6, 5.55 and 6.13 log cfu ml⁻¹, respectively of microbial count of ergo sample from Hawassa City, South Ethiopia. In addition, El-Malt LM, *et al.* (2013) in Qena

city, Egypt, indicated that, mean coliform count, *e.coli*, *psychrotrophic*, *entrococci*, *S.aureus*, yeast and mold count of 2.37x10³, 4.67x10³, 3.9x10⁴ (6.8x10³), 1.7x10⁴(2.0x10³), 8.5x10³(9.4x10²), and 1.4x10⁴(3.9x10²)count (cfu/ml) respectively in yoghurt sample.

Besides this, Mezgeb W. (2012) in Debre Libanose District, North Shewa zone Oromia region, reported that, the average total plate counts of milk samples were log 3.61 and 6.28 for udder and bulk milk tank at collection center respectively; the average coliform counts were log 1.76 and 4.17 for udder and bulk milk tank respectively. The two sampling points (udder and bulk milk at collection center) had significant differences (p≤0.001) in TPC and TCC counts. The average total plate counts and total coliform counts were higher in households that do not have clean barns, which did not wash udder or let calves suckle, not wash hands during milking and not use teat dip and towel.

The present finding were comparable with previous findings of (Tolessa and Asmamaw, 2017) in Asossa an average aerobic bacterial count of 7.08 log₁₀ (1.21x10⁷) to 7.41 log₁₀ cfu/ml (2.65x10⁷). Similarly, (Tassew and Seifu, 2011) at Bahir Dar Zuria with the overall mean of 7.58log₁₀cfu/ml, (Worku *et al.*,2012) who reported bacterial count from 7.36 -7.88 log₁₀ cfu/ml of raw cows' milk in Borana, Ethiopia and (Mosu *et al.*,2013) at selected dairy farms in Debre Zeit town, average aerobic bacterial count of 7.07log cfu/ml.

Moreover, this study was in line with study by (Endale *et al.*, 2013) where the overall mean bacterial count of cow's milk in Mekelle was 7.39log₁₀ cfu/ml at different points. However, the bacterial count obtained from current result was higher than that of work done by (Ashenafi and Beyene, 1994) reported as 6.32log₁₀cfu/ml, (Ombui *et al.*, 1995) reported as 5log₁₀ cfu/ml and (Bonfoh *et al.*, 2003) reported as 7log₁₀ cfu/ml). This is because of microbial load has highly associated with the hygienic condition practiced during harvesting to distribution process since the source of contamination is most of the time from the external environment than within animals.

Table 1: The mean (\pm SE) of microbial counts of ergo milk ($\text{Log}_{10}\text{CFU/ml}$)

Parameters	Locations		Overall mean (n=100)	P- value
	Urban(N=50)	Rural(N=50)		
<i>E.coli</i>	1922(3.28)	1848(3.26)	3770(3.77 \pm 0.53)	P<0.000
<i>Staphylococcus</i>	2346(3.37)	2223(3.34)	4570(4.57 \pm 0.83)	P>0.05
<i>Salmonella</i>	2045(3.31)	2463(3.39)	4508 (4.508 \pm 0.58)	P>0.000
<i>Yeast</i>	107(2.03)	91(1.96)	198(1.98 \pm 0.42)	P<0.05
<i>Mould</i>	71(1.85)	54(1.73)	125(1.25 \pm 1.14)	P<0.006

Table 2. Determination of Salmonella count/ ml of ergo (log 10 cfu/ml)

Woreda	Categories	Sample N=60	Microbial count (N=4508)	Overall mean		P value
				$\text{Log}_{10}\text{CFU/ml}$		
Abrahamo	Urban	15	1014	3.00	3.35	P<0.000
	Rural	15	1225	3.08		
Ura	Urban	15	1031	3.01	3.36	
	Rural	15	1238	3.09		

(As shown in Table 2, the mean *Salmonella* count was $4,508 \times 10^4$; Urban count= 2.045×10^6 ; rural count= 2.463×10^6).

Table 3. Total Staphylococcus count/ ml of ergo sample (log10 cfu/ml)

Woreda	Categories	Sample (N=60)	Mean Count N=4570	Overall mean		P - value
				$\text{Log}_{10}\text{CFU/ml}$		
Abrahamo	Urban	15	1228	3.089	3.37	P<0.000
	Rural	15	1150	3.06		
Ura	Urban	15	1118	3.05	3.34	
	Rural	15	1073	3.03		

(As shown in Table 3, the mean *Staphylococci* count was $4,570 \times 10^4$; Urban count= 2.346×10^6 ; rural count= 2.223×10^6).

Table 4. Total *E.coli* count / ml of ergo (log₁₀CFU/ml)

Woreda	Categories	Sample (N=60)	Mean Count (n=3770)	Overall mean		P value
				Log 10 CFU/ml		
Abrahamo	Urban	15	952	2.97	3.26	P<0.000
	Rural	15	903	2.95		
Ura	Urban	15	970	2.98	3.28	
	Rural	15	945	2.97		

(As shown in Table 4, the mean *E.coli* count was $3,770 \times 10^4$; Urban *E.coli* count= 1.922×10^6 ; rural *E.coli* count= 1.848×10^6).

Table 5. Determination of Yeast and Mold count of ergo milk (log₁₀CFU/ml)

Categories		Sample(N=60)		Overall mean		P –value
		No.	Count	Log ₁₀ cfu/ml		
Yeast		30	100			P<0.05
Abrahamo	Urban	15	54	2.02	2.29	
	Rural	15	46	1.96		
Mould		30	62			
Ura	Urban	15	35	1.85		
	Rural	15	27	1.73		

(As indicated above Table 5, the mean yeast and mold count was 162×10^4 cfu/ml; Urban fungus count= 89×10^4 ; rural fungus count= 73×10^4).

3.3 CHEMICAL QUALITY OF THE ERGO SAMPLES

Table 6 indicated that, the overall mean fat, protein, lactose, solids-not-fat (SNF), salt, density, and water content of the ergo samples as The mean value of fat, protein, lactose and solids-not-fat, salt, density, water contents were 4.81 ± 3.57 , 2.63 ± 0.97 , 3.78 ± 1.32 , 6.35 ± 2.34 , 0.53 ± 0.207 , 22.68 ± 9.74 , and 17.32 ± 26.77 respectively. The mean values of fat and protein in this study were higher than the Ethiopian Standard (ES, 2009) value of 4.5% and 3.20%, respectively. However, fat and protein values obtained in the present study were within the accepted range of 2.5 to 6.0% and 2.9 to 5.0% for fat and protein respectively (D. Belay & G. P. J. Janssen, 2014).

All the chemical composition values of ergo milk observed in the present study were higher than the

earlier findings of Veronique *et al.* (2013), who reported fat, protein, lactose and solids-not-fat (SNF) values of 4.36, 2.65, 3.96 and 7.22, respectively in urban dairy farms in Jimma. However, the results of the values of fat and SNF obtained in the current study were lower than the findings of Alganesh *et al.* (2009), who reported 6.05% fat and 8.22% SNF, and also lower than the previous report of Zelalem *et al.* (2010) who indicated 5.43 and 8.43% of fat and SNF, respectively. The variation in ergo composition values between the current and previous findings may be due to breed, nutritional status and health of cows, particularly that of udder health. As Table 9 indicated, the chemical composition of each ergo sample in the urban and rural areas were investigated and there was significant effect with respect to production channels such as vendors, producers, collectors and consumers (P<0.05).

Table 6: Chemical composition of ergo in urban and rural areas

Parameters	N	Minimum	Maximum	Mean \pm SD	t -value	p>/t/
Fat (%)	100	0.0	12.11	4.81 \pm 3.57	0.60	0.55
Protein (%)	100	0.6	4.0	2.63 \pm 0.97	0.53	0.59
Solid- not fat (%)	100	1.6	9.3	6.35 \pm 2.34.	0.16	0.87
Density (%)	100	6.7	34.1	22.68 \pm 9.74	0.39	0.69
Freezing point (%)	100	0.087	0.75	0.43 \pm 0.19	0.38	0.71
Lactose (%)	100	0.9	5.3	3.78 \pm 1.32	-0.30	0.76
Salt (%)	100	0.1	0.8	0.53 \pm 0.21	-0.46	0.65
Water (%)	100	0.1	82.2	17.32 \pm 26.8	2.16	0.035
pH (%)	100	3.85	6.43	4.91 \pm 1.11	-0.68	0.50

3.3.1. PH Level of Ergo

The average pH value of ergo sample collected from ergo production channels in the present study were between 6.02 and 3.8, respectively. The minimum pH value of ergo sample was 3.85 while the maximum value was 6.43 as Table 6 above indicated in the urban and rural areas. The current pH value is comparable with results reported by Rahel (Nebiyu R., 2008) that was, the least pH value observed in the ergo sample was 6.44, while in ergo it was 3.80, which was collected from Wolayita Zone.

Consistently, kidist FW *et al.*, (2015) in Addis Ababa on ergo indicated that, the mean pH value of homemade Ergo was 4.07 and the mean pH value for Mama flavored yoghurt was 3.52. And similarly, our result was found to be comparable with (ZelalemY, 2007) which reported the pH of Ergo samples around 4.02. Besides this, the author indicated, the mean pH values of Sholla yoghurt and Mama non-flavored yoghurt of 3.83 and 4.06, respectively. Younus *et al.*, (2002) reported that the pH value for market yoghurt was above 4.4, which is higher than results indicated in this study. The current result was higher than the pH (4.4) obtained from yoghurt produced from soya beans milk reported by (Akpan, *et al.*, 2007). In fact, as the pH of the products decrease the tetratable acidity increase (Akabanda *et al.*, 2010).

3.4. PHYSICAL QUALITY OF ERGO SAMPLES

The present study has highlighted the handling practices and microbial qualities of ergo 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 ergo producers used refrigerator after the ergo ferments. The handling of milk and ergo during transportation, storage and processing were generally poor. This was common particularly for ergo shops who take milk from multiple farms and those who do not follow strict sanitary

practices. According to Van Kessel *et al.* (2004), the use of insufficient and poor quality water for cleaning of milk handling equipment's can result in milk residues on equipment surfaces that provide nutrients for the growth and multiplication of bacteria that can then contaminate the milk.

Physical characteristic of ergo samples collected from the study area were shown in Table7. Of all the ergo milk samples tested, the majority (75%) had yellow white, which implies fermentation of ergo and might be exposed to contamination. Among the milk samples, the highest percentage (75%) had normal flavor, while 10% of the sample had off-flavor. The majority (85%) of the milk samples had normal texture (free flowing liquid), while three milk samples had thin texture, which might be due to high percentage of water, type of feed consumed prior to milking or breed of the milking cows maintained by the farms. The mean specific gravity of raw milk samples collected from urban dairy farms in this study was 1.027 \pm 0.00, ranging from 1.023 to 1.030. Comparably, Muluken A. *et al.*, (2016) in Ethiopia indicated, appearance (7.6), texture (7.6), color (7.0) and flavor (6.7) ergo character in cow.

The average specific gravity of the present study was slightly lower than the values of specific gravity of 1.030 reported by Alganesh *et al.* (2007) in eastern wellega, Ethiopia. The normal specific gravity of ergo milk ranges from 1.028 to 1.033 (FAO, 2013). Higher ergo milk specific gravity of about 1.035 and lower than normal value (1.020) are indicative of fat skimming off and the addition of water (Belay & G. P. J. Janssens, 2014).

Comparably, Muluken A. *et al.*, (2016) in his study stated that, the mean for sensory attributes of ergo cow's milk relatively a yellow color, and this color may probably reflected in the ergo product. And were similar in their judgments about the appearance and flavor of cows ergo i.e. the additives acts to improve the unacceptable flavor. Consistently, Kidist FW *et al.*, (2015) in Addis Ababa noted that, moisture content of

homemade and non-flavored yoghurt was above 96%; while the moisture content of flavored yoghurt was found lower than this value. In addition, Mbaeyi and Anyanwu, (2010) indicated that, the lower moisture content of flavored yoghurt sample could possibly associated with the addition of starch, pectin (found naturally in fruit), and/or gelatin that create thickness and creaminess.

Table 7: Physical quality of ergo samples collected from Study areas

Physical parameters	Number of samples	Quality	%
Color	75	Normal	75
	15	Light	15
	10	White	10
Flavor	75	Normal	75
	15	Sweet	15
	10	Off-flavor	10
Texture	85	Normal	85
	15	Thin	15
Specific gravity, mean \pm SD 1.27 \pm 0.00			

4. CONCLUSION AND RECOMMENDATIONS

The present study concluded that the quality of ergo milk received from the urban and rural locations were inadequate, as harmful microorganisms such as *Staphylococcus*, *E.coli*, *salmonella*, *yeast* and *mould* were found in high concentrations in the samples. *Staphylococcus*, *salmonella* and *E.coli* contaminated will render the milk unsafe for human consumption since many of these germs will cause illness and intoxication. As result of the current study's findings, more stringent preventive measures may be required following the identification of the conditions, that cause milk contamination. The magnitude of the problems of high microbial load merits more public health attention and comprehensive studies from milk productions to consumptions, as well as holistic preventive strategies to protect against unsafe milk and ensure that milk remains free of pathogens and spoilage microorganism. So that based on the above conclusion, the following recommendations are forwarded;

- Training programs must be provided on best practice of ergo milk such as handling, sanitary, hygiene of ergo and/or fermented milk for handlers,

- Raising the level of awareness for producers, consumers, and traders or collectors, vendor of restaurant and cafeterias;
- *Salmonella*, *e.coli*, *Staphylococcus*, *fungus* are resistant to most common drugs, attention should be taken in selecting antimicrobials in treating infection both in animals and human being, so drug selection should be based on antimicrobial susceptibility test;
- The degree of the risk of consumption of ergo contaminated with *bacteria* should be assessed;
- The use of standardized procedures and applications like good hygienic practice, handling of ergo, cleaning of ergo containers and raw milks at producing origin,
- Further study should be conducted to identify the source of contamination,

5. REFERENCES

- [1]. Abebe, B., Zelalem, Y. and Ajebu, N. (2012). Hygienic and microbial quality of raw whole cow's milk produced in *Ezhad* district of the Gurage zone, Southern Ethiopia. *Wudpecker J. of Agri. Res.* Vol. 1(11), pp. 459 – 465.
- [2]. Alganesh, T., L.N. Ofodile and B. Fekadu, 2007. Microbial quality and chemical composition of raw whole milk from Horro cattle in East wollega
- [3]. Alganesh, T., Ofodile, L.N. and Fekadu, B. (2009). Microbial quality and chemical composition of raw whole milk from Horro cattle in East Wollega, Ethiopia. *Microb. Qual. Chem. Compos.* 1: 1-8.
- [4]. Ali, A.A. (2011). Isolation and Identification of Lactic Acid Bacteria Isolated from Traditional Drinking Yoghurt in Khartoum State, Sudan. *Curr. Res. Bacter.* 4(1): 16-22.
- [5]. Almaz, G., Alemu, F., Kelbessa, U. and Berhanu, A.G. (1999). Microbiological aspects of 'Ergo' ('Ititu') fermentation. *SINET: Ethiop. J. Sci.* 22: 283-289.
- [6]. Asmahan A. Ali and Warda S. Abdelgadir (2011): Incidence of *Escherichia coli* in raw cow's milk in Khartoum state, *British Journal of Dairy Sciences* 2(1):23-26 pp 23-26.
- [7]. Behnke, R., & Metaferia, F. (2011). *The Contribution of Livestock to the Ethiopian Economy-Part II*, IGAD Livestock Policy Initiative (LPI) Working Paper.
- [8]. BenishangulGumuz Regional State Assosa Town Administration Agriculture Directive, 2020. Annual action performance report unpublished
- [9]. Berg VD, JCT (1988): Dairy technology in the tropics and sub tropics center for agricultural

- supplicating and documentation, Wageningen, Netherlands.
- [11]. Bezahegn Abebe, 2019. Cattle Fattening Practices in West Hararge: Potentials and Constraints of Beef Cattle Production in Oromia Regional State, Ethiopia. *Journal of Agricultural Science*; Vol. 11, No. 8; 2019 ISSN 1916-9752 E-ISSN 1916-9760
- [12]. Biruk-Kemaw, 2018. Livestock Production Constraints, Opportunities and its Determinant in Assosa District, Benishangul-Gumuz Regional State, Western Ethiopia. *Journal of Biology, Agriculture and Healthcare* www.iiste.org ISSN 2224-3208 (Paper) ISSN 2225-093X (Online) Vol.8, No.3, 2018
- [13]. Biruktawit, SH. (2016). Physicochemical Properties And Microbial Quality Of Cow Milk Collected From Selected Sub-city Of Addis Ababa, Ethiopia. M.Sc Thesis, *Addis Ababa University, College of veterinary Medicine and Agriculture, Department of Animal Production Studies*.
- [14]. Central Statistical Agency, (CSA). (2013). Agricultural Sample Survey, Volume II: Report on Livestock and livestock characteristics (Private peasant holdings). Statistical Bulletin 570. Addis Ababa: Federal Democratic Republic of Ethiopia.
- [15]. Coorevits, A., Jonghe, De, Vandroemme V., Reekmans, J., Heyrman, R., Messens, J., De Vos, W., P. Heyndrickx, M. (2008). Comparative analysis of the diversity of aerobic-spore-forming bacteria in raw milk from organic and conventional dairy farms. *System. Appl. Microbiol.*, 31(2):126-40. doi: 10.1016/j.syapm.2008.03.002. Epub 2008 Apr 10.
- [16]. CLIS (Clinical and Laboratory Standards Institute), 2009. Methods for dilution Antimicrobial Susceptibility Testing for Bacteria that grew aerobically. Approved Standard M7-A10. Wayne, PA: Clinical and Laboratory Standards Institute.
- [17]. CSA (Central Statistical Authority), 2020. Projection made From 2007 Population and Census out in Ethiopia
- [18]. CSA, 2017. Agricultural sample survey. Federal Democratic Republic of Ethiopia Central Statistical Agency. Private Peasant Holdings. Statistical Bulletin 570, Addis Ababa, Ethiopia, April, 2011.
- [19]. CSA, 2017/18. Agricultural sample survey report on livestock and livestock characteristics. Addis Ababa, Ethiopia.
- [20]. DuPont, H.L, 2007. The growing threat of foodborne bacterial entero- pathogens of animal origin. *Clin. Infect. Dis.* 45, 1353–1361.
- [21]. EAS, 2007. *Raw Cow Milk – Specification*. East African Community (EAC 67:2007) standard.
- [22]. EMDIDI (Ethiopian Meat and Dairy Industry Development Institute), 2016. Ethiopian Agro-Industry Strategy Meat Industry Sub-Sector Strategic Plan. Addis Ababa, Ethiopia.
- [23]. Ethiopian Agro-Industry Strategy Meat Industry Sub- Sector Strategic Plan, 2016. The Federal democratic republic of Ethiopia-ministry of industry (Final Version).
- [24]. El-Malt LM, Abdel Hameed KG and Mohammed AS (2013): Microbiological evaluation of yoghurt products in Qena city, Egypt, *Vet World* 6(7).
- [25]. Nebiyu R (2008) Traditional and improved milk and milk products handling practices and compositional and microbial quality of milk and buttermilk in delbo watershed of wolayta zone, Ethiopia.
- [26]. ET ISO 707. (2012). Milk and milk products - Guidance on sampling. FDRE, EDDICT OF GOVERNMENT, Ethiopian Standards Agency, 1st Edition.
- [27]. FAO. (2013). Milk and dairy products in human nutrition. Ellen, M., Anthony, B. and Deirdre, Mc. (eds). Food and Agriculture Organization of the United Nations, Rome.
- [28]. Fernandese, R. (2009). Microbiology handbook, Dairy products. Leatherhead food International Limited. ISBN: 978-1-905224-62-3, Cambridge, UK.
- [29]. Frisvad, J.C., Skouboe, P., Samson, R.A. (2005). Taxonomic comparison of three different groups of aflatoxin producers and a new efficient producer of aflatoxin B sterigmatocystin and 3-O-methylsterigmatocystin, *Aspergillus rambellii* sp. nov. *Syst. Appl. Microbiol.*, 28, 442–453.
- [30]. FAO, 2019. Livestock health, livelihoods and the environment in Ethiopia. An integrated analysis. Rome. 108 pp.
- [31]. Federal Democratic Republic of Ethiopia National Meteorology Agency Benishangul Gumuz Region Meteorology Service Center, 2020. Annual action performance report unpublished.
- [32]. Kadariya, J., Smith, T.C., Thapaliya, D., 2014. *Staphylococcus aureus* and *staphylococcal* food-borne disease: an ongoing challenge in public health. *BioMed Res. Int.* 2014.
- [33]. Kiiyukia, C. (2003). Laboratory manual of food microbiology for Ethiopian health and nutrition research institute. Addis Ababa.
- [34]. Mezgeb Workiye (2012): Study on milk chemical composition, bacteriological` quality and handling practices in Debre Libanose District, North Shewa zone Oromia region. Msc thesis, Addis Ababa University.
- [35]. Mogessie, A. (1995): Microbial development and some chemical changes during the making of

- 'Ergo', a traditional Ethiopian fermented milk. *Bull. Anim. Health Prod. Afr.* 43: 171-176.
- [36]. Muluken Asfaw, Admasu Erika Cione, Stefano Aquaro (2016): Microbiological Characteristics and Physico-chemical Parameters of Fermented Milk Product Ergo-A Traditional Yogurt Product of Ethiopia, Department of Pharmacy and Health Sciences and Nutrition, Università della Calabria, Food Science and Quality Management ,Vol.49, 2016;pp 42-45.
- [37]. National Committee for Clinical Laboratory Standards (NCCLS), 2005. Performance standards for antimicrobial susceptibility testing; 15th informational supplement. NCCLS document M100- S10., Wanyne, and pa.
- [38]. Pal M, 2012. Raw meat poses public health risks. *The Ethiopian Herald*.2-3.
- [39]. Quinin P.J., Carter ME., Markey BK and Carter GR, 2002. *Veterinary microbiology* 6th edition, Wolfe publishing. Pp 21-67.
- [40]. Rota, C., Yanguela, J., Blanco, D., Carraminana, J., Arino, A. and Herrera, A, 1996. High prevalence of multiple resistances to antibiotics in 144 *Listeria* isolates from Spanish dairy and meat products. *J. FO. Prot.*,59: 938–943.
- [41]. Shambel Bekele, 2017. Impacts of Climate Change on Livestock Production: A Review Journal of Natural Sciences Research www.iiste.org ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online) Vol.7, No.8, 2017
- [42]. Shapiro, B. I., Gebru, G., Desta, S., Negassa, A., Nigussie, K., Aboset, G. and Mechal, H, 2015. Ethiopia livestock master plan. ILRI Project Report. Nairobi, Kenya: International Livestock Research Institute (ILRI).
- [43]. Stephen B. Smith, Takafumi Gotoh and Paul L. Greenwood, 2018. Current situation and future prospects for global beef production: overview of special issue. Vol. 31, No. 7:927-932 July 2018.
- [44]. Tadesse BT, 2014. Prevalence of the Major Infectious Animal Diseases Affecting Livestock Trade Industry in Ethiopia. *J of Bio Agri and Health care*.4 (17): 76-82.
- [45]. Tegegn, A., and Soboka, S. (2020). Assessment of microbial and physicochemical qualities of cow milk in selected districts of Borena Zone, Oromia Regional State, Ethiopia. *Global Journal of Animal Scientific Research*, 8(3), 72-92.
- [46]. Teshome G., and Tesfaye A., (2016). Physicochemical properties and microbial quality of raw cow milk produced by small holders in Bench Maji- Zone, South western Ethiopia, *Food science and quality management*, vol. 54., pp 47-54.
- [47]. Teshome G., Beyene F., Eshetu M., (2014). Handling practices and Microbial Quality of raw cow's milk produced and marketed in Shashemene Town, Southern Ethiopia. *Int.J. Ag.Ric. Soil Sci, Vol2(9)*:153-162.
- [48]. Thrusfield, M, 2007. *Veterinary Epidemiology*. 3rd ed. Blackwell Science Ltd., London, England, Pp. 228-245.
- [49]. Tilahun, H, Schmidt, E. (2012). Spatial Analysis of Livestock Production Patterns in Ethiopia. ESSP II Working Paper 44. Addis Ababa, Ethiopia: International Food Policy Research Institute/Ethiopia Strategy Support Program II.
- [50]. Weldearegay, H., Zelalem, Y. and Yosef, T.G. (2012). Hygienic practices and microbiological quality of raw milk produced under different farm size in Hawassa, southern Ethiopia. *Agri. Res. Revie.* 1(4): 132 – 142
- [51]. Weldaragay H, Yilma Z, Tekle – Giorgis Y. (2012). Hygienic practices and microbiological quality of raw milk produced under different farm size in Hawassa, southern Ethiopia. *woodpecker J Agric Res Rev.* ;4:132-42.
- [52]. Yilma, Z. (2012). Microbial Properties of Ethiopian Marketed Milk and Milk Products and Associated Critical Points of Contamination: An Epidemiological Perspective, *Epidemiology Insights*, Dr. Maria De Lourdes Ribeiro De Souza Da Cunha (Ed.), ISBN: 978-953-51-0565-7.
- [53]. Yigrem S, Welearegay H (2015) : Assessment of Microbial Quality and Safety of a Traditional Fermented Milk- 'Irgo', Collected from Hawassa City, South Ethiopia. *J Food Process Technol* 6: 43.
- [54]. Zelalem, Y. (2009). Microbial properties of Ethiopian marketed milk and milk products and associated critical points of contamination: An epidemiological perspective. East Africa Dairy Development (EADD) Program, Addis Ababa, Ethiopia 4
- [55]. Van Kessel SJ, Karns JS, Gorski L (2004): Prevalence of salmonellae, *listeria monocytogenes*, and fecal coliforms in Bulk tank milk on US dairies. *American Dairy Science Association, J. Dairy Sci* 87: 2822-2830
- [56]. Walstra P, Wouters M TJM, Geurts TJ (2006): *Dairy Science and Technology*, CRC press. London

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