Microbial Load and Occurrence of Bacterial Pathogens in *Ayib* (Ethiopian Cottage Cheese) Marketed in Hawassa City, Southern Ethiopia

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Abstract: *Ayib*, a traditional Ethiopian cottage cheese, is a popular dairy product with a significant role in the country's diet and culture. However, despite its high nutritional quality, it is susceptible to microbial spoilage and transmission of foodborne pathogens. Therefore, this study aimed to assess the microbial quality and safety of *ayib* samples sourced from small-scale vendors in the open markets and a commercial dairy plant in Hawassa, Ethiopia. The microbial quality and safety of 50 *ayib* samples (250 g per sample) consisting of 25 samples from randomly selected small-scale vendors and 25 from retail outlets of a commercial dairy in Hawassa. The quality and safety of the *ayib* samples were assessed based on aerobic mesophilic bacterial count (AMBC), total coliform count (TCC), staphylococcal count (SC), yeast and mold count (YMC), and detection of *Salmonella* and *Escherichia coli* using standard plate count and phenotypic biochemical tests. The mean AMBC, TCC, SC, and YMC values of samples from the small-scale vendors in $\log_{10}$CFU/g were 8.26, 5.38, 4.96, and 6.88, respectively. The respective parameters for the *ayib* samples from the dairy plant were 7.55, 4.56, 3.91, and 5.42 in $\log_{10}$ CFU/g. The aerobic mesophilic bacteria count in the *ayib* samples from both sources were dominated by *Staphylococcus*, *Bacillus*, and *Pseudomonas*. *Salmonella* was detected at higher frequencies (48%) in samples from small-scale vendors than in the dairy plant (28%). These findings indicated that *ayib* samples from both sources had a microbial load higher than the recommended limit set by international standards. The study highlighted the potential risks associated with *ayib* consumption, particularly from small-scale vendors. It emphasizes the need for improved food safety practices and regulations to ensure the safety and quality of *ayib* in the production and supply chain.

Keywords: *Ayib, Dairy plant, Hawassa, Microbial safety, Small-scale vendors*

Introduction

Ayib (Ethiopian cottage cheese) is one of the most commonly produced and consumed dairy products in Ethiopia (Almaz *et al.,* 2001). *Ayib* consists 79 % water, 15 % protein, 2 % fat, 1% ash, and 3% soluble milk (Mogessie, 1992). The typical traditional *ayib* production process involves the fermentation of raw milk by lactic acid bacteria (LAB) that inherently enter raw milk without the use of defined starter cultures to initiate the process. The resulting coagulated product, Ergo, is then churned or agitated to separate the butter. The defatted sour milk (Arera) is then used as a precursor for *ayib* making. During the processing of *ayib*, arera is placed in a clay pot and heated with a slow fire at approximately 40-70°C until a distinct curd mass forms (Mogessie, 2002). When the curd and whey are separated, the heating is stopped and the content of the pot is allowed to cool. After that, the whey is drained off through a fine mesh cloth or sieve and the cheese curd is kept in a clean bowl or in an airtight container (Figure 1).

The production process in commercial dairy plants is more or less the same with remarkable differences in scale, equipment, and use of pasteurized skimmed milk as raw material. Briefly, after removal of the cream, the raw milk is pasteurized batch-wise and allowed to undergo fermentation by surviving resident lactic acid bacteria at ambient temperature for at least 24 h. The coagulated milk is then heated to approximately 75 ^{0}C until distinct curd forms. The whey is drained and the curd is pressed with a cheesecloth to remove any remaining whey. Finally, the resulting *ayib* is packaged and stored in a cold room until it is distributed to the retail market. Approximately 10 kg of *ayib* is obtained from 100 L of raw milk (personal observations and discussion with the production manager).

Traditional soft curd cottage cheese (*ayib*) is quite acidic, with average pH values ranging from 3.7-4.6 (Zelalem *et al.*, 2007). Despite its low pH, *ayib* has a shelf life of 2-3 days due to its high-water activity and vulnerability to spoilage molds (Mogessie, 1990). The shelf-life may be improved by adding salt and spices, smoking the containers with herbs (olive), or reducing the high moisture content (Abebe *et al.,* 2014). These techniques are particularly useful during fasting months, such as the lent, to preserve the surplus *ayib* and sale it in festive times. The use of traditional preservation methods for *ayib* is also practiced in places where market access is limited for surplus product. However, unhygienic handling and display of products in the open market is widespread and can lead to contamination and

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transmission of food-borne pathogens In addition to the potential for pathogen transmission and intoxication, some microorganisms can also contribute to a decline in the appearance and sensory properties of *ayib*, which affects its marketability (Tekletsadik, and Tsige, 2011).

The microbial quality and safety of *ayib* products from small-scale vendors varies depending on the level of hygiene exercised during production and marketing (Zelalem, 2010; Alganesh, 2017). Mogessie (1994) reported the isolation and identification of several spoilage and pathogenic bacteria from *ayib* samples, including *Pseudomonas*, and *Salmonella*. In another study from open market in Hawassa, Mogessie (2002) also reported that more than 90% of the *ayib* samples had a mean aerobic mesophilic bacterial count (AMBC) exceeding 8 $\log_{10}$ CFU/g, and more than 75% of the samples had yeast counts at levels of 7$\log_{10}$ CFU/g or higher. These findings indicate that the safety of *ayib*, in terms of the presence of pathogenic microorganisms, is a significant concern. This is particularly relevant for *ayib* produced by traditional small-scale producers under generally unsanitary conditions and using unpasteurized raw milk. It highlights the importance of implementing proper hygiene practices and regulatory interventions to ensure the microbial safety of marketed *ayib* products. In the absence of surveillance and enforced public health food laws, periodic cross sectional investigation of market samples may serve as an early warning system to prevent foodborne diseases outbreak.

In the case of commercial dairy plants, the pasteurization process is presumed to destroy pathogens and greatly reduces spoilage microflora, and ensures safety of products (Lewis and Deeth, 2008). However, problems may still arise due to the use of substandard-quality raw milk, which may contain a very high initial microbial load. The majority of dairy plants in Ethiopia use raw milk collected from small-scale farmers in rural and suburban areas where the basic amenities of food safety, such as refrigerated storage are lacking. A recent survey showed that good manufacturing practices (GMP) was not uniformly applied in milk processing plants in Ethiopia (Mulugeta *et al.*, 2020). When the initial microbial load of raw milk is too high, regular pasteurization procedures may not be sufficient to destroy pathogens and reduce microbial spoilage (Abraham *et al.,* 2021).

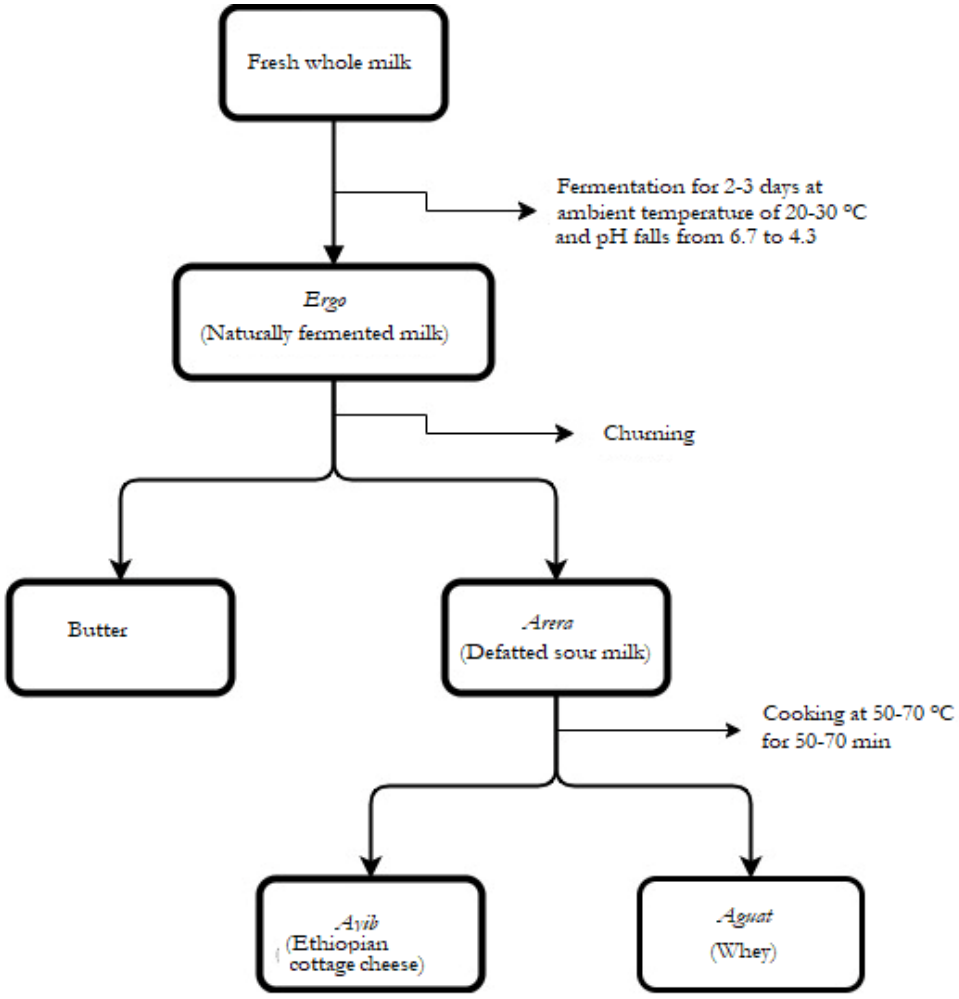

Figure 1. Flow diagram of Ethiopian cottage cheese (*ayib*) making process.

The above-mentioned problem is further aggravated by the lack of development and weak enforcement of food laws and regulatory systems (Melese and Melese 2015). Additionally, there is a limited analytical capacity at the national and regional levels (Abdi *et al.,* 2020). The existing food laws in Ethiopia are outdated and primarily focus on end-product inspection, without a proactive approach to raw material and process control. It is crucial to revise and evaluate the food laws in line with the requirements of the current trends in the expansion of hotels, tourism, and trade. This revision should be guided by data inputs from scientific research. Previous studies conducted in Ethiopia have predominantly focused on the analysis and reporting of the microbial quality of *ayib* from traditional small-scale vendors (Mogessie, 1990; Mogessie, 1994; Seifu *et al*., 2013; Eyassu, 2013; Mebrat *et al*., 2016; Jermen *et al*., 2016; Abdi, 2022).

While the hygienic status and safety of *ayib* from small-scale vendors are questionable, the relative cost is much more affordable compared to *ayib* produced in modern dairy plants. As a result, many *ayib* lovers prefer products from open markets. Although documented reports on this matter are scarce, there is potential for food-borne disease outbreaks resulting from the consumption of contaminated *ayib* products. If such outbreaks occur, the repercussions can be far-reaching, risking public health and having economic consequences for the hotel and tourism industry. Therefore, this study aimed to assess the microbial quality and safety of *ayib* samples from small-scale vendors in the open market and a retail outlet of a commercial dairy in Hawassa.

Materials and Methods

Description of the Study Area

The study was conducted in Hawassa city, the administrative center of the Sidama Regional State in Ethiopia. Located about 275km south of Addis Ababa, Hawassa has an elevation of 1697m above sea level and is situated within coordinates 7° 3'N and 38° 28' E/7.050° N 38.467° E. The city is known for its tourist attractions, including a Rift Valley Lake and diverse cultures. It is home to the dairy plant included in this study. Although the plant has a capacity to handle up to 20,000 liters daily, it is currently processing approximately 2500 liters of raw milk per day. The dairy plant did not keep its own dairy cows and the raw milk for processing is collected from small-scale farmers in the neighboring rural areas (Abraham *et al.,* 2021).

Hawassa city has a potential for dairy farming and production, benefiting from access to various water sources such as deep wells, tap water, and Lake Hawassa, which provide drinking water for animals (Haile *et al*., 2012). The main feed resources for cows in the city include crop residues like stover (especially maize), grass hay, and industrial byproducts such as spent grains or 'Attela' from brewing residue (Melaku, 2019). According to some estimates, the average daily milk yield in households is 13.3 liters for small farms, 51.5 liters for medium farms, and 81.4 liters for large farms (Haile *et al*., 2012). However, the shortage of animal feeds is a significant limiting factor for dairy production, followed by constraints related to housing, milking, waste disposal, expansion, and animal disease incidence in Hawassa (Melaku, 2019).

Design of the Study

A cross-sectional study was conducted based on laboratory analysis of the microbial quality and safety of *ayib* samples collected from small-scale vendors in the open market and from a commercial dairy plant in Hawassa city, southern Ethiopia during the period from December 2018 to November 2019.

Sampling Procedure, Sample Size, and Sample Collection

A non-probabilistic, convenient sampling approach was followed to include a total of 50 *ayib* samples (250 g per sample) consisting of 25 from small-scale vendors in an open market and 25 *ayib* samples from the retail outlet of a commercial dairy plant (Supermarket). The *ayib* samples were purchased from five small-scale vendors in the open market, Arogegebeya (old market) located in the Mehal Ketema subcity, while different batches of *ayib* samples from a commercial dairy plant were purchased from supermarkets in Hawassa. Five *ayib* samples were analyzed per week, thus, requiring ten weeks for all samples. All *ayib* samples from small-scale vendors in the open market were collected in sterile stainless-steel lunch boxes while those from the dairy plant were purchased in plastic bags (packed in the factory). All samples were placed in an ice box and transported to the Food Science and Technology Laboratory, College of Agriculture, Hawassa University, Hawassa. In case of delay, the samples were kept at refrigerator temperature (4 ^{O}C) before analysis.

Laboratory Analysis

Preparation of microbiological media and reagents: All microbiological culture media and reagents used in this study were prepared following the recommended procedures according to the manufacturer's instructions (HiMedia Laboratories, India and Oxoid, UK).

Preparation of decimal dilution: For all samples, *ayib* was mixed thoroughly using a sterile spatula, and 10 g of the sample was weighed aseptically into a sterile bottle containing 90 mL of sterile buffered peptone water. From this bottle, a further tenfold serial dilution was prepared by the transfer of 1 mL aliquots using a sterile pipette into test tubes containing 9 mL of buffered peptone water and labeled as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. Vortex mixing was performed between each transfer into tubes to ensure uniform homogenization (Andrew, 1992).

Aerobic mesophilic bacterial count (AMBC): From the appropriate decimal dilutions prepared above, 0.1mL aliquots were aseptically transferred into, separately labeled plates of plate count agar (PCA) (HiMedia, India)

in duplicates and spread plated with sterile bent glass rods. The bent glass rod was sterilized by immersing it in absolute ethanol and burning off the alcohol. The inoculated plates were incubated at 37°C for 48-72 hrs. At the end of the incubation, the colonies were counted using a Quebec dark field colony counter (Richert), and plates with 25-250 colonies were used to calculate the average AMBC in CFU/g (Andrew, 1992).

Total coliform count (TCC): Appropriate dilutions were inoculated by spread plating onto MacConkey agar (Oxoid, UK) in duplicates as described above for AMBC and incubated at 37^{O}C for 48 h. At the end of the incubation period, the colonies were counted using the Quebec colony counter (Richert), and plates with 25-250 colonies were used to calculate the average TCC (Andrew, 1992).

***Staphylococcus aureus* count (SC):** Appropriate dilutions were inoculated by spread plating on duplicate Mannitol salt agar (HiMedia) plates as described above forAMBC and incubated at 37 ^{o}C for 48h. At the end of the incubation period, plates with 25-250 typical yellow colonies were used to calculate the average SC in CFU/g.

Yeast and mold count (YMC): From an appropriate tenfold dilution, 0.1 mL aliquots were spread plated onto the surface of potato dextrose agar (PDA, Hi-media, India) supplemented with 1% (w/v) of chloramphenicol and tetracycline antibiotics in duplicate plates to selectively inhibit the growth of bacteria and other competing microorganisms (Tournas *et al.,* 2001). The plates were then incubated at room temperature (25°C) for 5 days. Finally, plates with 25-250 colonies were used to calculate the average YMC of the *ayib* samples in CFU/g.

Determination of the dominant aerobic, mesophilic bacteria (AMB): The dominant AMB were determined by following the FAO guideline (Andrew, 1992). Briefly, five to ten distinct colonies were individually selected from the countable plates used in the AMBC. These colonies were then purified through repeated sub-culturing on nutrient agar plates. After purification, the isolates were stored in 20% glycerol cryopreservation vials at -20°C until further characterization was conducted. Cryopreservation was performed by mixing 800 µL of the overnight nutrient broth culture of each isolate with 200 µL of sterile glycerol. The purified isolates were subsequently characterized and provisionally identified based on colony morphology, Gram staining, microscopic examination, and biochemical tests including catalase test, urease test, reaction on EMB agar, triple sugar iron agar (TSI), sulfide indole motility (SIM) medium, methyl red test, Voges-Proskauer test, and the utilization of citrate (Andrew, 1992).

Screening and detection of *Escherichia coli:* From countable plates used in the TCC above, five to ten distinct colonies were picked and purified by repeated sub-culturing. The purified isolates were streaked onto plates of eosin methylene blue agar (EMB) and subjected to the IMViC tests. Isolates that showed black colonies with green metallic sheen on EMB agar, positive for indole and methyl red test but negative for Vougus-Proskauer and citrate utilization test were identified as *E. coli* (Andrew, 1992).

Detection of *Salmonella*: To detect *Salmonella*, a loop full of the whole *ayib* sample were directly streaked separately onto the surface of *Salmonella-Shigella* agar (SSA). In order to resuscitate metabolically injured cells, streaking on the same media was also performed after overnight culture of 0.1 mL aliquots from the 1:10 dilution into a tube containing 5 mL of sterile nutrient broth. All plates were incubated at 37 ^{o}C for 48 h and at the end of the incubation typical colonies (black) were picked and purified by repeated sub-culturing. Purified presumptive isolates were subjected to selected biochemical tests for confirmation by inoculation into sulfide–indole–motility (SIM) agar, triple sugar iron (TSI), Urea agar, and Lysine iron agar (LIA). Isolates were presumptively identified as *Salmonella* based on results: gram-negative small rod, hydrogen sulfide positive, indole negative, motile, non-lactose fermenter, and lysine decarboxylase positive (Andrew, 1992).

pH

The pH of *ayib* samples from both small-scale vendors in the open market and a dairy plant was measured using a digital meter equipped with a glass electrode. The glass electrode was immersed in cheese samples that were homogenized with distilled water (Kariyawasam *et al*., 2019).

Data Analysis

All enumeration was done in duplicates by spread plating and values were transformed into a log unit for ease of manipulation. To calculate the average load from multiple plates, the following formulae was used (Maturin and Peeler, 2003; Abraham *et al.,* 2022):

$$N = \frac{\sum C}{(n_1 + 0.1n_2)\, d}$$

Where $\sum C$ is the sum of colonies counted on all the dishes retained; n_1 is the number of dishes retained in the first dilution; n_2 is the number of dishes retained in the second dilution; d is the dilution factor corresponding to the first dilution (Maturin and Peeler, 2003). Descriptive statistics were used to analyze and present the data in tabular form, including summary values such as the minimum, maximum, average, and standard deviation. The mean values of all microbial load parameters were compared between *ayib* samples obtained from small-scale vendors and those from the commercial dairy plant. This comparison was conducted using the student t-test, and any differences observed were considered statistically significant at a $p < 0.05$.

Results and Discussion

Aerobic Mesophilic Bacterial Count (AMBC)

The average AMBC of the *ayib* samples from small-scale vendors ranged from 7.27 to 8.50 $\log_{10}$ CFU/g with the mean value being 8.2 $\log_{10}$ CFU/ g (Table 1). The mean AMBC value in the present study is higher than the 6.13 $\log_{10}$ CFU/g of AMBC for *ayib* samples from Jimma (Tekletsadik and Tsige, 2011), and the range of 5-8 $\log_{10}$ CFU/g AMBC for *ayib* samples collected from North Shoa Districts (Jermen *et al.,* 2016). However, it is lower than the 8.844 $\log_{10}$ CFU/g AMBC for the *ayib* sample collected from southwest Ethiopia (Seifu *et al*., 2013). The mean AMBC value in the present study is comparable with the 8.3 $\log_{10}$CFU/g value reported by Haddad and Yamani (2017) for traditional soft white cheese collected in Jordan. Based on a similar study, Mogessie (1990) reported an average AMBC of 8 $\log_{10}$ CFU/g for *ayib* samples collected from the open market in Hawassa, which is also closer to the current finding. *Ayib* is a fermented food and according to the International Commission for Microbiological Specification of Foods (ICMSF, 2011), AMBC is not applicable to fermented foods. Nonetheless, high counts are suggestive of poor hygienic handling and storage conditions. A high AMBC count in *ayib* from small-scale vendors can be expected considering the poor infrastructure and hygienic practices exercised in the production chain (Mogessie, 1990). In general, small-scale household dairy processing in Ethiopia is characterized by a lack of clean and safe water for cleaning utensils and ignorance of basic food hygiene (Kebede *et al.,* 2020), which results in microbial contamination of the raw milk and its byproducts during processing, storage and transportation.

Table 1. The microbial load of *ayib* (Ethiopian cottage cheese) samples in log10 CFU/g and pH from small-scale vendors in the open market and a dairy plant in Hawassa city, 2019.

SN	***Ayib* from small scale vendors**					***Ayib* from the commercial dairy plant**				
	AMBC	**TCC**	**CS**	**YMC**	**pH**	**AMBC**	**TCC**	**CS**	**YMC**	**pH**
S1	8.21	4.21	5.2	7.43	4.30	7.28	ND	ND	6.26	3.94
S2	7.31	ND	ND	7.15	3.60	7.47	ND	ND	6.57	4.11
S3	8.09	7.68	6.14	7.54	4.58	7.27	ND	ND	6.32	4.04
S4	8.55	ND	4.91	7.36	3.91	7.27	4.43	ND	6.34	4.06
S5	8.31	ND	4.12	7.19	3.78	7.32	4.37	ND	6.59	4.05
S6	8.05	ND	5.03	6.63	3.80	7.48	ND	ND	6.51	4.09
S7	8.53	ND	6.44	8.98	3.82	7.27	ND	ND	6.29	4.02
S8	8.44	ND	4.72	7.15	3.75	8.50	5.39	ND	6.48	4.07
S9	8.64	ND	5.76	7.44	3.85	8.33	4.04	ND	6.33	4.00
S10	8.65	ND	6.02	7.43	3.92	7.48	ND	4.47	6.40	4.30
S11	8.51	ND	4.39	6.66	3.90	7.56	ND	ND	6.26	5.16
S12	7.34	ND	4.26	6.59	3.72	7.39	ND	3.48	ND	4.91
S13	7.31	ND	4.24	6.46	3.70	7.57	ND	3.30	ND	4.50
S14	8.33	ND	4.37	6.65	3.78	7.44	ND	4.27	6.38	4.20
S15	8.52	ND	4.81	6.77	3.91	7.45	ND	ND	6.59	4.40
S16	7.97	ND	4.72	6.71	3.74	8.22	ND	4.24	ND	4.46
S17	7.77	ND	4.73	6.6	3.76	7.37	ND	3.60	ND	4.38
S18	8.60	ND	5.28	6.04	3.86	7.37	ND	3.48	6.29	5.03
S19	8.62	5.17	4.62	6.38	4.00	7.54	ND	4.45	6.68	5.13
S20	8.71	5.38	5.15	6.04	4.06	8.22	ND	3.30	6.74	5.38
S21	8.24	5.23	4.58	6.50	3.90	7.60	ND	4.23	6.72	5.20
S22	8.30	5.27	4.97	6.57	3.93	7.30	ND	3.48	6.38	5.00
S23	8.35	4.88	4.48	6.29	3.94	7.29	ND	4.10	6.42	4.60
S24	8.51	5.32	5.10	6.7	3.96	7.32	ND	ND	6.51	5.08
S25	8.74	5.29	5.00	6.65	4.09	7.47	ND	4.47	6.52	5.03
Min	**7.31**	**0.00**	**0.00**	**6.04**	**3.6**	**7.27**	**0.00**	**0.00**	**0**	**3.94**
Max	**8.74**	**7.68**	**6.44**	**8.98**	**4.58**	**8.5**	**5.39**	**4.47**	**6.74**	**5.38**
Mean	**8.26**	**1.94**	**4.76**	**6.88**	**3.9**	**7.55**	**0.73**	**2.03**	**5.42**	**4.53**
Std	**0.43**	**2.69**	**1.16**	**0.62**	**0.2**	**0.36**	**1.72**	**2.02**	**2.42**	**0.48**

SN= Sample number; AMBC = Aerobic mesophilic bacterial count; TCC = Total coliform count; CS = Count for Staphylococcus aureus; YMC = Yeast and mold count; ND = Not detectable; Min = Minimum; Max = Maximum; Std = Standard deviation.

Considering the AMBC of the *ayib* samples from the commercial dairy, it ranged from 7.27 $\log_{10}$ CFU/g to 8.50 $\log_{10}$ CFU/g, with a mean value of 7.55 $\log_{10}$ CFU/g (Table 1). This value was significantly lower than that for the *ayib* samples from the small-scale vendors (Table 2). This difference may be explained by the obvious reason that *ayib* from small-scale vendors is traditionally made from raw unpasteurized milk whereas the preparation of *ayib* in modern dairy farms involves prior pasteurization of the milk. Proper pasteurization of milk destroys pathogens and greatly reduces spoilage microbes (Owusu-Kwarteng *et al.,* 2020). According to European standards (EC, 1992), ready-to-eat foods should have an AMBC $\leq 10^5$ CFU/g. This criterion is not applicable to fermented foods like *ayib*. However, values greater than this in ready-to-eat food generally indicate poor hygienic handling. The mean AMBC value of the *ayib* samples from the dairy plant in the present study is higher than the 6.13 $\log_{10}$ CFU/ g of AMBC for *ayib* samples from Jimma (Tekletsadik and Tsige, 2011). This finding is logically unexpected since the *ayib* samples studied in Jimma were collected from small-scale vendors. The discrepancy may be explained by the poor quality or high microbial load of the raw milk used for processing by the plant. As stated in the previous sections, most dairy plants in Ethiopia do not have their own dairy cow-rearing lot and depend on the collection of raw milk from rural areas and the lack of strict follow-up of good manufacturing practices (Mulugeta *et al*., 2020). Besides, factors such as post-pasteurization contamination of milk during cheese production, improper handling, and issues with preservation at retail outlets can contribute to higher AMBC in *ayib* from commercial dairy plants (Abraham *et al.,* 2021).

Total Coliform Count (TCC)

The average total coliform count of *ayib* samples from the small-scale vendors ranged from not detectable (ND) to 7.68 $\log_{10}$ CFU/g with the mean being 1.94 $\log_{10}$ CFU/g (Table 1). Several earlier works carried out in different parts of Ethiopia reported coliform counts of *ayib* samples that ranged between 2 $\log_{10}$ CFU/g and 5.68 $\log_{10}$ CFU/g (Almaz *et al*., 2001; Seifu *et al*., 2013; Jermen *et al*., 2016; Abdi, 2022). The mean TCC of the *ayib* samples in the present study (1.94 $\log_{10}$ CFU/g) is lower than this range. The observed differences in the mean TCC may be attributed to differences in the source of samples and hygienic handling exercised (Zelalem *et al.,* 2005). The mean TCC value in the present study is also lower than the 3.93 $\log_{10}$ CFU/g of TCC reported for raw cottage cheese (Kareish) from Egypt (Mohamed *et al.,* 2019).

The average total coliform count (TCC) for the *ayib* samples from the dairy plant ranged from ND to 5.39 $\log_{10}$ CFU/g and the mean was 0.73 $\log_{10}$ CFU/g (Table 1). This value was lower than that of *ayib* samples from the small-scale vendors. However, the observed difference was not statistically significant (Table 2). This might be attributed to post-pasteurization contamination, improper handling, and problems in preservation at retail outlets of the *ayib* from the commercial plant (Abraham *et al*., 2021). The mean TCC of the *ayib* samples from the dairy plant in the present study is comparable with the report of Mohamed et al. (2019) who found a 0.31$\log_{10}$ CFU g/1TCC for pasteurized cottage cheese (Kareish) collected from Egypt.

The majority of the *ayib* samples from the small-scale vendors in the open market (64%) and those from the dairy plant retail outlet (84%) showed no detectable coliform bacteria and are in compliance with the recommended (<10 CFU/g) acceptable level of TCC (ICMSF, 2011). This might be due to the combination of the low pH of the products (Table 1) or high acidity (Trmčić *et al*., 2016), the effect of pasteurization of the raw milk, and the curd cooking temperature during *ayib* production. Coliform bacteria are generally very sensitive to heat treatment and are not known to survive ultra-high temperature (UHT) pasteurization temperature (Walstra *et al.,* 2006).

Table 2. Comparison of the mean microbial load (AMBC, TCC, SC, YMC) and pH values for the *ayib* samples from small-scale vendors (n=25) and a commercial dairy plant (n=25) in Hawassa, southern Ethiopia.

Microbial load parameters	T	df	P-value (2-tailed)	Mean difference	Standard error difference	95% confidence interval of difference	
						Lower	Upper
AMBC	6.37	48	0.00*	0.71	0.112	0.49	0.94
TCC	1.89	48	0.065	1.21	0.638	-0.08	2.49
SC	5.85	48	0.00*	2.73	0.466	1.79	3.66
YMC	2.91	48	0.005*	1.45	0.499	0.45	2.46
pH	-6	48	0.00*	-0.62	0.104	-0.83	-0.42

** Indicates mean microbial load between the small-scale vendors and the dairy plant differ significantly at p-value < 0.05; AMBC = Aerobic mesophilic bacterial count; TCC = Total coliform count; SC = Staphylococcal count; YMC = Yeast and mold count; df = Degrees of freedom; T = Student T-test.*

The Staphylococcus aureus Count (SC)

The mean CS of the *ayib* samples from the small-scale vendors ranged from not detectable (ND) to 6.44 $\log_{10}$ CFU/g and the mean was 4.76 $\log_{10}$ CFU/g (Table 1). The mean SC of the *ayib* samples from the small-scale vendors in the present study is lower than that reported for *ayib* samples from Jimma (5.39 $\log_{10}$ CFU g/1) (Tekletsadik and Tsige, 2011) and 6.5$\log_{10}$ CFU/g of traditional soft white cheese reported by Haddad and Yamani (2017) from Jordan. However, it is higher than

2.94log_{10} CFU/g of raw Kareish cheese samples reported from Egypt (Mohamed *et al.*, 2019). The mean CS of *ayib* samples from the small-scale vendors in the present study is comparable with the 4.98log_{10} CFU/g of *ayib* samples from different value chains in the Oromia region of Ethiopia (Abdi, 2022). It is also comparable to the CS reported, ranging from 2.7 to 4.3 log_{10} CFU/g for *ayib* samples from the North Showa District in Ethiopia (Jermen *et al.,* 2016). Seven (28%) of the *ayib* samples from small-scale vendors had mean CS higher than the recommended maximum of 4 log_{10} CFU/g for raw milk cheese (ICMSF, 2011). When the count of *S. aureus* exceeds 4 log_{10} CFU/g of ready-to-consume food like *ayib*, it is considered unacceptable and potentially hazardous (Masoud *et al.,* 2012). In the present study, the ability of the isolates to produce enterotoxins was not assessed. Elsewhere, several studies have reported the results of experiments on the survival and growth potential of enterotoxigenic *S. aureus* during the production of different types of cheese (Al-Nabuls *et al.,* 2020; Lai *et al.,* 2020). Based on these studies, several factors were reported to influence the behavior of *S. aureus* in cheese made from raw unpasteurized milk, including background microbiota of the raw milk, starter culture, pH, and salt among others. The consensus is that when *S. aureus* count exceeds 5 log_{10} CFU/g, staphylococcal enterotoxin (SE) production is likely to occur (Masoud *et al.,* 2012). According to international standards, coagulase-positive staphylococci must not be equal to or exceed 5 log units per gram in any one sample of raw milk cheese (ICMSF, 2011).

With regard to the *ayib* samples from the commercial dairy plant, the mean CS ranged between ND and 4.47 log10 CFU/g, with an average value of 2.03 log10 CFU/g (Table 1). This mean value was significantly lower ($P<0.05$) than that of the *ayib* samples from the small-scale vendors (Table 2). As mentioned earlier, these differences are likely attributed to variations in hygienic handling practices and the fact that *ayib* produced in modern dairy plants is made from pasteurized milk and undergoes standardized curd cooking temperatures. Unlike the mean CS of *ayib* samples from the dairy plant (2.03 log_{10} CFU/g) in the present study, Mohamed *et al.* (2019) reported the absence of *S. aureus* in pasteurized Kareish cheese samples from Egypt.

The majority of the *ayib* samples from the dairy plant (13 or 52%) had mean CS that exceeded the recommended maximum of 2 log_{10} CFU/g for fresh pasteurized milk cheese (ICMSF, 2011). Such high mean SC is considered to increase the risk of intoxication from enterotoxigenic (ET) strains (EC, 2003). Unlike the enterotoxins they produce, staphylococci are generally heat labile, and destroyed by regular UHT pasteurization of milk (Murphy *et al.,* 2016). Hence, their presence in heat-treated milk and milk products indicates post-processing contamination. This may also be due to the growth of the limited number of survivors of pasteurization and curd cooking in a viable but nonculturable (VBNC) state due to larger loads in raw milk (Abraham *et al.,* 2021). Bacteria in the VBNC state can resuscitate and proliferate upon return to favorable growth conditions (Ding *et al.,* 2017). Post-processing contamination and growth of contaminants may also be possible due to temperature abuse during transport and storage in the retail market (Abraham *et al.,* 2021).

Yeast and Mold Count (YMC)

The mean YMC of the *ayib* samples from the small-scale vendors and the dairy plant showed a statistically significant difference at $P<0.05$(Table 2). The mean YMC of the *ayib* samples from the small-scale vendors was 6.88 log_{10} CFU/g (Table 1). This value is higher than the 3.1 to 4 log_{10} CFU/g YMC reported for *ayib* samples from North Shoa (Mamo *et al.,* 2016) and the 2-3 log_{10} CFU/g of low moisture ('metata') *ayib* samples from West Gojam (Eyassu, 2013), Ethiopia. Elsewhere, Haddad and Yamani (2017) reported a mean YMC of 3. log_{10} CFU/g for traditional soft white cheese samples collected in Jordan, which is much lower than that of the *ayib* samples from the small-scale vendors in the present study. Likewise, Mohamed *et al.* (2019) reported a mean YMC of 3.17 log_{10} CFU/g for raw Kareish cheese samples collected in Egypt. Yeast and molds are important spoilage organisms in low pH foods like *ayib* owing to their wider pH optima and proteolytic and lipolytic activity (Copetti, 2019). When the *ayib* product is stored for a prolonged period (which is the case in times of fasting months), the danger of growth and elaboration of mycotoxins from mycotoxigenic molds may not be uncommon (Kure and Skaar, 2019).

Concerning the *ayib* samples from the commercial dairy plant, the average yeast and mold count (YMC) ranged from not detectable to 6.74 log10 CFU/g, with an average value of 5.42 log10 CFU/g (Table 1). While this YMC value is lower than that of the *ayib* samples from small-scale vendors, it is considerably higher than the reported YMC value of 1.16 log10 CFU/g for pasteurized Kareish cheese samples from Egypt (Mohamed *et al.,* 2019). Although certain types of molds are intentionally used in the cheese ripening process to enhance flavor and taste, most, wild fungi are more likely to cause spoilage (Copetti, 2019). As mentioned before, the growth of molds and yeasts in *ayib* is influenced by its high acidity and extended storage duration, as these microorganisms have a broader range of optimal pH levels. Fungi, in general, have a wider tolerance for pH, water activity, and other physiological conditions compared to bacteria (Jay *et al.,* 2005). However, unlike *ayib* products from small-scale vendors, *ayib* produced by the commercial dairy plant is not subject to longer transit time in transportation and may not undergo prolonged storage, as it is manufactured and supplied to urban consumers based on market demand. The shorter duration of transport and storage minimize the growth of molds and yeasts in the *ayib* from the commercial dairy plant.

The Dominant Aerobic Mesophilic Bacteria (AMB)

By selecting morphologically distinct colonies from countable PCA plates used in AMBC, a total of 132 AMB were isolated. Of these, 79 were isolated from the *ayib* samples from small-scale vendors in the open market and 53 from the *ayib* samples of the dairy plant. Colony morphology, Gram staining, microscopy, and selected biochemical tests allowed putative identification of the bacteria into four genera. Accordingly, the dominant genera from the small-scale vendors were *Staphylococcus* (25/79 or 31.65%), *Bacillus* (23/79 or 29.11%), and *Pseudomonas* (14/79 or 17.72%). Unidentified members of the family *Enterobacteriaceae* (10/79 or 12.66%) represented the next less frequent mesophilic bacteria, while seven isolates (8.86%) were unidentified Gram-positive bacteria (Figure 2).

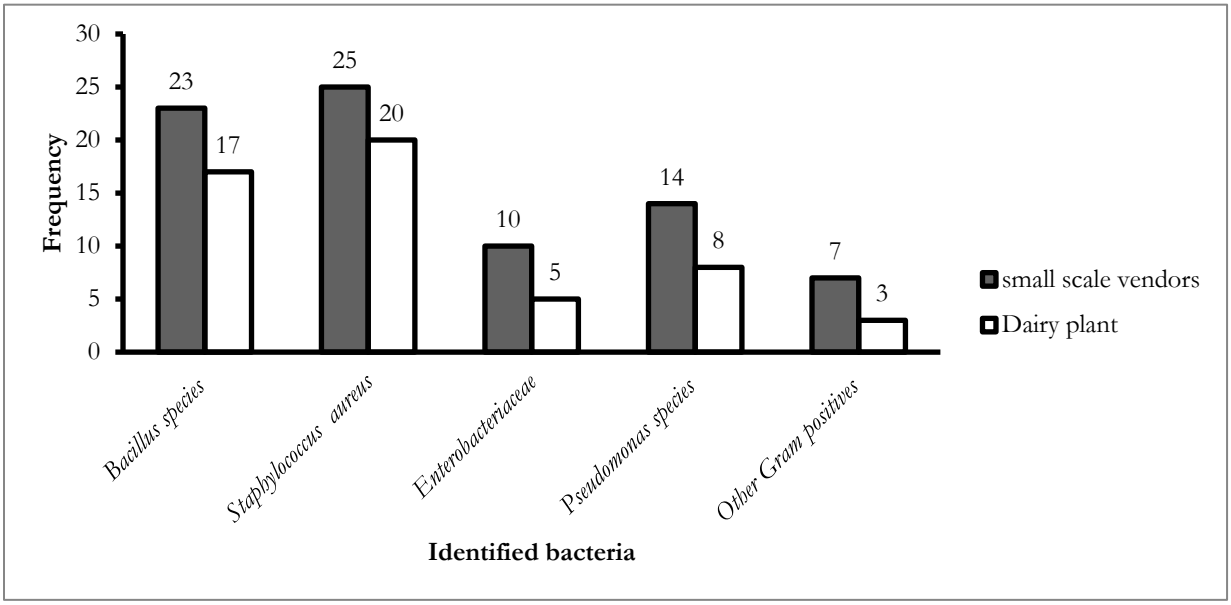

Figure 2. Frequency distribution of the dominant aerobic mesophilic bacteria isolated from *ayib* samples collected from small-scale vendors in open market and a modern dairy plant in Hawassa city.

Interestingly, the same bacterial genera, *Staphylococcus* (20/53 or 37.74%), *Bacillus* (17/53 or 30.10%), and *Pseudomonas* (8/53 or 15.10%) constituted the dominant mesophilic aerobic bacteria in the *ayib* samples from the dairy plant (Figure 2). Likewise, unidentified members of the family *Enterobacteriaceae* (5/53 or 9.43%) represented the next less frequent mesophilic bacteria, while three isolates (5.66%) remained unidentified Gram-positive bacteria. All three dominant AMB genera are known to have members that are frequently incriminated in spoilage of foods owing to their proteolytic and lipolytic activity as well as their ability to grow at low temperature as in the case of *Pseudomonas*. Therefore, keeping the quality of *ayib* from both sources is likely to be compromised (Jay *et al*., 2005).

In a similar study of *ayib* samples from small-scale vendors in Hawassa, Mogessie (1994) also reported the isolation of several spoilage and pathogenic bacterial genera similar to the present study, including, *Microbacterium*, *Brevibacterium* spp, member of the family *Enterobacteriaceae*, *Pseudomonas* spp, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Salmonella Typhimurium*, *Salmonella Enteritidis, Salmonella Infantis*. Likewise, Eyassu (2013) reported *Bacillus cereus*, *Lactobacilli*, heat-resistant *Staphylococcus* species, *Klebsiella* species, *Escherichia coli*, *Enterobacter* species, and the yeast *Klyuvera* species in moist *ayib* sourced from open markets in Amhara Region of Ethiopia. Although several different bacteria may contaminate cottage cheese in the production and market chain, the dominant ones that will grow and cause spoilage are governed by the prevailing intrinsic and extrinsic parameters (Modise, 2014).

The Occurrence of Escherichia coli and Salmonella

Escherichia coli was encountered in 48% (12 of 25) of the *ayib* samples from small-scale vendors in the open market and in 28% (7 of 25) of the samples from the dairy plant (Figure 3). The occurrence of *E. coli* in the *ayib* samples from both sources in the present study is higher than the 20.67% for *ayib* samples from southwest Ethiopia (Seifu *et al.,* 2013). The occurrence of *E. coli* in the *ayib* samples from both sources in the present study were far lower than the reported 86.7% in raw Kareish cheese by Mohamed *et al.* (2019) and 81% in Kareish cheese samples by Awad (2016), both in Egypt. The higher occurrence of *E. coli* in the *ayib* samples from small-scale vendors is probably due to the use of unpasteurized raw milk, low curd cooking temperature treatment, and unhygienic handling. Although *E. coli* is generally considered a harmless commensal of the gastrointestinal tract of humans and other warm-blooded animals, several pathovars are also known to cause disease in humans (Croxen *et al.,* 2013). It is often used as an indicator of sanitary quality and an index organism for the occurrence of other enteric pathogens in food and beverages (Martin *et al.,* 2016). *E. coli* is heat

labile (Mostert and Jooste, 2002) and its presence in the *ayib* samples indicates post-curd fecal contamination and a general lack of sanitary handling.

Regarding *Salmonella*, it was detected in eight of the 25 (32%) *ayib* samples from small-scale vendors and four of the 25 (16%) from the dairy plant (Figure 3). In contrast with the present study, Mebrat *et al.* (2016) reported the absence of *Salmonella* spp. in *ayib* samples from Gondar city. The absence of salmonellae, was also reported by Awad (2016) in Kareish cheese samples from Egypt. On the other hand, Omar *et al.* (2018) reported the occurrence of salmonellae in 16% of fresh cheese collected from street vendors in Egypt, which is twofold lower than the occurrence of *Salmonella* in *ayib* samples from the small-scale vendors in the present study. Salmonellae are heat-sensitive bacterial pathogens and do not survive the curd cooking temperature during *ayib* making (Kebede *et al.,* 2020). Therefore, their occurrence in the *ayib* samples indicates post-processing contamination. According to the recommended standards, salmonellae should not be detectable in any sample of ready-to-eat foods like *ayib* (EC,1992).

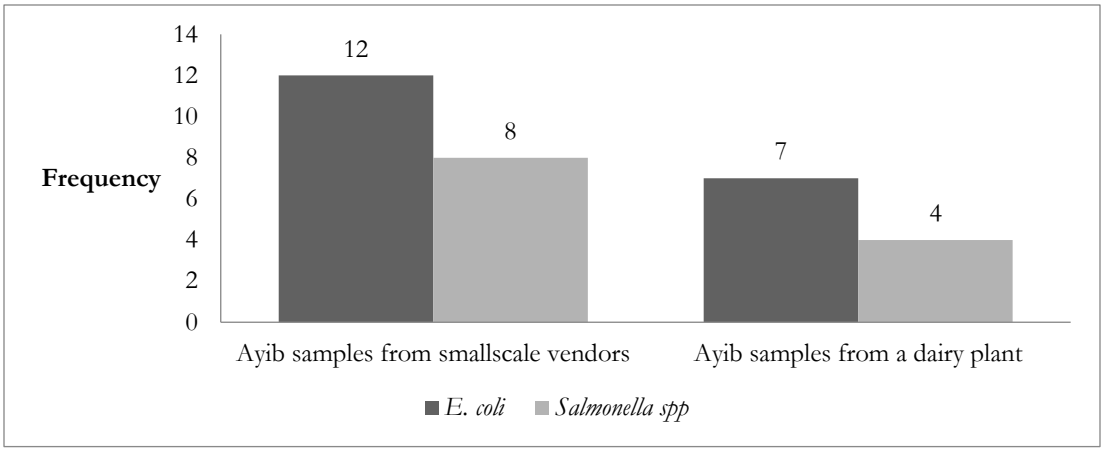

Figure 3. The occurrence of *Salmonella* and *Escherichia coli* in milk samples from small-scale vendors and a dairy plant in Hawassa city, southern Ethiopia, 2019.

Conclusion

In conclusion, this study assessed the microbial quality and safety of *ayib* samples from small-scale vendors and a commercial dairy plant in Hawassa, Ethiopia. The results revealed that the *ayib* samples from the commercial dairy processing plant had better microbial quality compared to those from the small-scale vendors. However, both sources showed high microbial load values, exceeding the recommended standards for ready-to-eat foods. The detection of *Salmonella* and *E. coli* in both *ayib* samples from the small-scale vendors and the dairy plant indicates poor sanitary conditions during the preparation and handling of the products. The dominant bacterial genera found in the *ayib* samples from both sources were *Staphylococcus*, *Bacillus*, and *Pseudomonas*. These findings highlight the potential risks associated with *ayib* consumption, particularly from small-scale vendors. It emphasizes the need for improved food safety practices, including revising good manufacturing and hygienic practices in the dairy plant, as well as providing basic education in food hygiene to small-scale vendors. The regional health office, Food and Drug Administration office, and other stakeholders should collaborate to ensure the production of safe and high-quality *ayib* in the market. It is important to note that this study used small samples and was limited to phenotypic and biochemical tests for the detection and identification of bacterial genera. Therefore, future works should envisage a more comprehensive sampling and the application of better microbiological detection and identification methods to reveal the true magnitude of the problem.

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Conflict of Interests

The authors declare that they have no competing interests.

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