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HYGIENIC PRACTICES AND QUALITY OF TRADITIONAL *ERGO* IN DAIRY PRODUCT SELLING SHOPS IN HOLETA AND ADDIS ALEM TOWNS

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A THESIS SUBMITTED TO FEDRAL TECHNICAL AND VOCATIONAL TRAINING INSTITUTE, FACULTY OF AGRO-PROCESSING TECHNOLOGY (DEPARTMENT OF DAIRY PROCESSING TECHNOLOGY)

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN DAIRY PROCESSING TECHNOLOGY

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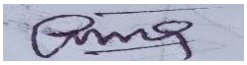
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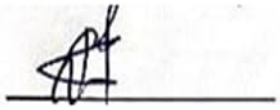
DECLARATION

I, the undersigned entitled **"Hygienic Practices Quality of Traditional *Irgo* in Dairy Product Selling Shop in Holeta and Addis Alem towns"** declare that the thesis comprises my own work. In compliance with internationally accepted practices, I have dually acknowledged and refereed all materials used in this work. I understand that non-adherence to the principles of academic honesty and integrity, misrepresentation/ fabrication of any idea/data/fact/source was constitute sufficient ground for disciplinary action by the university and can also evoke penal action from the sources which have not been properly cited or acknowledged.

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APPROVAL SHEET

This is to certify that the thesis entitled **"Hygienic Practices Quality of Traditional *Irgo* in Dairy Product Selling Shop in Holeta and Addis Alem towns"** submitted in partial fulfillment of the requirements for the degree of **Master's** with specialization in Dairy Processing Technology**,** the Graduate Program of the **Department of Dairy Processing** Technology**,** and has been carried out by **Atomsa Gonfa Muleta** under my/our supervision. Therefore I/we recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to the department for defense.



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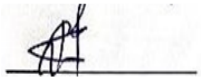
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FACULTY OF AGRO-PROCESSING TECHNOLOGY

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HYGIENIC PRACTICES AND QUALITY OF TRADITIONAL ERGO IN DAIRY PRODUCT SELLING SHOPS IN HOLETA AND ADDIS ALEM TOWNS

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ABBRIVATIONS AND ACRONOMYS

CC	Coliform Count
EBC	Enterobacteriaceae Count
LAB	Lactic Acid Bacteria
LBC	Lipolytic Bacteria Count
LSD	Least Significance Difference
PCA	Plate Count Agar
SMA	Skim Milk Agar
SMP	Skim Milk Powder
SPC	Slandered Plate Count
SPSS	Statistical Package for Social Science
TA	Titaretable Acidity
TBC	Total Bacterial Count
VRBA	Violet Red Bile Agar
VRBGA	Violet Red Bile Glucose Agar
YMC	Yeast And Mold Count

ABSTRACT

This study investigated the hygiene practice, and quality of traditional "Irgo," in Dairy product, sold in Holeta and Addis Alem towns, Ethiopia. A survey of 80 Irgo sellers revealed that the majority were male, over 40 years old, and had basic education. While all sellers reported hand washing before Irgo preparation, 25% did not use soap and towels. Most sellers cleaned their shops more than once a week and practiced daily utensil cleaning, often involving heat treatment of water and smoking with traditional plants. Physicochemical analysis showed that Irgo from Addis Alem had higher total solids and protein content, while Holeta samples had slightly higher fat content. pH and titratable acidity were within acceptable ranges, though moisture and total solid levels exceeded some standards. Microbiological analysis indicated variations in bacterial counts between the two locations, with Addis Alem showing higher total bacterial and coliform counts. Yeast and mold counts exceeded established standards in both areas. However, Escherichia coli was not detected in any samples. Sensory evaluation revealed that Holeta Irgo samples had higher overall acceptability, particularly in appearance, aroma, and taste, while Addis Alem samples scored higher in texture. The study concludes that while Irgo production generally adheres to some hygienic practices, improvements are needed to meet microbiological standards, particularly regarding yeast and mold contamination. Recommendations include stricter hygiene measures, regular monitoring by authorities, and educational programs for Irgo producers to enhance product safety and quality.

Key Terms: ***Irgo, hygienic, Physicochemical, Microbiological, Sensory evaluation***

TABLE OF CONTENTS

DECLARATION iii
APPROVAL SHEET iv
AKNOWLEDGEMENTS vi
ABBRIVATIONS AND ACRONOMYS vii
ABSTRACT viii
TABLE OF CONTENTS ix
LIST OF TABLES xi
1. INTRODUCTION 1
1.1. Background 1
1.2. Statement of the Problems 4
1.3. Objectives of the Study 4
1.3.1. General objective 4
To assess the hygienic practices and quality attributes of *Irgo* collected from different dairy product shops in Holeta and Addis Alem town. 4
1.3.2. Specific objectives 4
1.4. Basic Research Questions 5
1.5. Significance of the Study 5
1.6. Scopes of the Study 5
1.7. Limitation of the Study 5
2.1. Fermented Milk Production in Ethiopia 6
2.2. Handling Practices of Milk and *Irgo* 7
2.3. Physicochemical Properties of *Irgo* 8
2.3.1. Protein 8
2.3.2. Fat 9
2.3.4. Total solid 10
2.3.5. Ash 10
2.3.6. Titratable acidity 11
2.3.7. pH 11
2.3.8. Carbohydrate 11
2.4. Microbial Quality of *Irgo* 12
2.4.1. Total bacteria count 12
2.4.2. Coliform Count in *Irgo* 13

2.4.3. Yeast and mould count 14
2.4.4. Escherichia coli (E.coli) 14
2.4.5. Microbiological specifications of yoghurt 15
2.5. Quality and Safety of a Fermented Product 16
2.6. Sensory Evaluation 18
3. MATERIALS AND METHODS 19
3.1. Description of the Study Area 19
3.2. Study Design 20
3.3. Sampling Techniques and Sample Size Determination 20
3.4. Data Collection 20
3.4.1. Analysis of physicochemical composition of *irgo* 21
3.4.2. Microbial quality analysis 24
3.4.3. Sensory evaluation 26
3.5. Statistical Analysis 26
4. RESULTS AND DISCUSSION 27
4.1. Socio-economic Characteristics 27
4.2. Hand Washing Practices 28
4.3. Shop Cleaning Frequency 29
4.4. Water Sources and Treatment 29
4.5. Physico-chemical Composition of *Irgo* 31
4.6. Microbiological Quality of *Irgo* 34
4.7. Sensory of Evaluation of *Irgo* 37
5. CONCLUSION AND RECOMMENDATIONS 38
5.1. Conclusion 38
5.2. Recommendations 39
REFERENCES 40

LIST OF TABLES

Table 1. Socio economic characteristics of the respondents in the study area 27
Table 2: Hand washing practices in the study areas.....28
Table 3. Shop cleaning frequency in the study areas 29
Table 4: Water source, treatment and cleaning practices of milk collecting and Irgo preparing tools 30
Table 5: Physico-chemical composition of Irgo in the study areas (%) 33
Table 6: Microbiological quality of *Irgo* (log10cfu/ml) in the study areas.....35
Table 7: Mean and standard deviation (Mean ±SD) for Evaluation of sensory quality of Irgo samples from Holeta and Addis Alem town 37

1. INTRODUCTION

1.1. Background

Milk is lacteal secretion produced by mammalian animals to feed their young (REF). It is highly nutritious, considered as complete food and important part of daily diet for mothers as well as for growing children (Upadhyay *et al.,* 2014 and Javaid *et al.*, 2009). According to the European Commission (2010), functional food, besides its basic nutritional value, improves health benefits and reduces the risk of diseases.

A functional food can be a natural food or one to which components have been added, removed, or modified (Roberfroid, 2002). Traditional dairy foods can be considered functional foods as they have nutritional value and more importantly, they impart health benefits. Among those, one of the functional milk-based products is yogurt. Yogurt is one of the most popular products originating and is widely consumed worldwide (Playne *et al.*, 2003).

Irgo is a traditional fermented milk product, which has some resemblance to yogurt and is made by natural fermentation of milk under ambient temperature, with no defined starter cultures used to initiate the fermentation processes (Zahara & Zinewi, 2015; Bereda *et al.*, 2014). The rural peoples in Ethiopia are producing fermented milk by traditional methods. The major fermented milk products produced by smallholder farmers by traditional methods include "*Irgo*" (fermented sour milk), "*Ititu*" (Fermented milk curd), "*Kibe*" (traditional butter), "*Neter kibe"* (spiced butter), "*Ayib*" (cottage cheese), "*Arerra*" (Sour defatted milk), and "*Aguat"* (whey) (Esayas *et al*., 2008).

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 (Bereda *et al.,* 2014) It is popular and is consumed in all parts of Ethiopia by all age categories of people (Zahara & Zinewi, 2015).

Irgo has been described as a balanced food, containing almost all the nutrients present in milk but in a more digestible form (Admasu & Cione, 2016). It is a good source of iodine, calcium, phosphorus, zinc, riboflavin, vitamin B5 and vitamin B12. It is also nutritionally rich in protein, molybdenum and pantothenic acid. It has also several health benefits like probiotic effect it balances normal intestinal flora, antibiotic and antimicrobial effects, it was scientifically proved that *Irgo* was effective in prevention of trace element toxifications. According to Almaz *et al.* (2001) report, Irgo can be stored for 15-20 days depending on the storage temperature.

Understanding the physicochemical properties of *Irgo* is essential for quality control, product development, and ensuring consumer satisfaction. These properties are influenced by various factors such as the milk source, starter cultures used in fermentation, processing techniques, and any additional ingredients added to the final product. Product quality and consumer satisfaction are important for increasing the demand of various types of yoghurt.

The increase in the per capita annual consumption of yoghurt in the majority of the countries has been attributed to increased knowledge regarding the health benefits of yoghurt (Küçüköner and Tarakçi, 2003; Van de Water and Naiyanetr, 2008). This product also contribute to the protein requirements of the indigenous consumers, because the nutritional value of milk proteins is well preserved during the fermentation process (Gursoy *et al*., 2010) and lowering the pH of food products through fermentation is a form of food preservation. Both the caseins and the whey proteins in yogurt are rich source of Amino acids (93%) and high in nitrogen availability.

Amino acids like proline and glycine are present in free form and higher contents in yogurt than in milk (Damian, 2013). Although much of the yogurt sold in industrialized countries is produced from skimmed milk, traditional *Irgo* has always contained some 3-4 g per100 g milk fat; indeed concentrated yogurt or Greek-style yogurts will contain 9-10 g per100 g fat (Tamine and Robinsion, 1999).

In the case of natural yogurt, a number of mono and disaccharides are present in trace amounts, but lactose remains the dominant sugar in natural yogurt; even after fermentation, the product may contain some 4-5 g per 100 g lactose. The reason for this residue is that the

processed milk is often fortified to 14-16 g per 100 g total solids (i.e. up to about 8 g per 100 g lactose), so that the lactose content of the end product is little different from normal milk (Tamine and Robinsion,2000). However, the effect that these apparently identical levels of lactose can have on people who are so called lactose intolerant (Damian, 2013).

Irgo is highly perishable and can lose its quality within a short period if not handled under hygienic conditions (Mekonine and Mengistu, 2017). The handling and safety of milk and milk products is of great concern around the world, this is especially true in the developing country where production of milk and various dairy products takes place under unsanitary condition and poor production sale must consider the health of consumer (Almaz *et al.*, 2001).

The microorganisms principally encountered in the dairy industry are bacteria, yeasts, molds and viruses. Some of the bacteria (lactic acid bacteria) are useful in milk processing, causing milk to sour naturally. However, milk can also contain pathogenic bacteria, such as *Salmonella spp., Staphylococcus aureus, Escherichia coli, Mycobacterium tuberculosis, Listeria spp., Brucella spp* (Lingathurai and Vellathurai, 2010).

The presence of these pathogenic bacteria in milk decrease the shelf-life of the product which lower the grade and unsafe products (Motawee & Saleh, 2016), emerged as major public health concerns, especially for those individuals who still drink raw milk (Lingathurai and Vellathurai, 2010). In Ethiopia, milk and milk products handling practice is below the standard due to insufficient pre-milking handling practices like washing udder with clean water, cleaning milking barn, drying the udder with individual towel, washing of milkers' hands and milking utensils, using of poor quality and non-boiled water for cleaning of udder.

In addition, insufficient post handling practice like poor hygiene of milk equipment and storage containers, prolonged storage, transportation and retailing practices predispose the milk to microbial contamination (Tsedey and Asrat, 2015; Fufa *et al.*, 2019). Hence, the present study was designed to assess the hygienic practices and quality attributes of *Irgo* collected from different dairy product shops in Holeta and Addis Alem town, oromia regional state, Ethiopia.

1.2. Statement of the Problems

Most of food-related illnesses are caused by improper food storage conditions, and inadequate hygienic procedures in Ethiopia, where the production of milk and various dairy products often occurs under unsanitary conditions and the consumption of raw milk and milk product is common (Gebremedhin *et al.,* 2022). The key to producing safe and high-quality foods for consumption is minimizing microbiological contamination.

Previously, only a few study were conducted on nutritional value and microbiological characterization of raw milk, however with potential of the area have on dairy product specifically *Ergo* the nutritional and microbiological quality and safety of traditional ergo in dairy product selling shop specifically in Holeta and Addis Alem town is not studied yet. Therefore, this research finding certainly would be provide tangible information on physicochemical and microbiological quality and safety of traditional *Irgo* in dairy product selling shop in the Holeta and Addis Alem town of west Shoa zone of Oromia regional state of Ethiopia.

1.3. Objectives of the Study

1.3.1. General objective

To assess the hygienic practices and quality attributes of *Irgo* collected from different dairy product shops in Holeta and Addis Alem town.

1.3.2. Specific objectives

- To assess the hygienic practices *Irgo* in dairy product selling shop in the study areas.
- To evaluate the physicochemical composition of traditional *Irgo* in dairy product selling shop in the study areas.
- To evaluate the microbiological quality and safety of *Irgo* in dairy product selling shop in the study areas.

1.4. Basic Research Questions

1. How does the hygienic practices of *Irgo* in dairy product selling shops in Holeta and Addis Alem town?
2. How does the physicochemical composition of *Irgo* in dairy product selling shops in Holeta and Addis Alem town?
3. How does the microbiological quality and safety of *Irgo* in dairy product selling shops in Holeta and Addis Alem town?

1.5. Significance of the Study

Studying of microbiological and nutritional quality of *Irgo* is not questionable in terms of human health because it is highly consuming fermented milk product in Ethiopia. This study was focus on assessing the hygienic practices and quality attributes of *Irgo* in the study areas. The finding would be contributed to show and evaluating the handling practices, physicochemical and microbiological quality and safety of *Irgo*. Finally, the best practice of milk handling was proposed.

1.6. Scopes of the Study

The study is limited in terms of coverage and depth owing to time and financial resources availability. This study aspires to offer a comprehensive overview of handling practices, physicochemical characteristics, and microbiological quality parameters in *Irgo*. By exploring these dimensions in depth, the research aims to contribute to the advancement of quality assurance practices in the dairy industry, ultimately enhancing the safety, quality, and consumer satisfaction of *Irgo*.

1.7. Limitation of the Study

This research work is limited to determine the physicochemical, microbiological quality and safety of traditional *Irgo* in dairy product selling shops in Holeta and Addis Alem towns. Hence, generalization of findings of the study may be limited to the specific district considered in the undertaking. A large sample size from traditional *Irgo* seller within and outside Holeta and Addis Alem towns could have allowed for generalizations of the findings.

2. LITERATURE REVIEW

2.1. Fermented Milk Production in Ethiopia

Fermented dairy products such as cream, butter, yogurt, kefir, and cheese have a rich history of production and consumption worldwide for centuries. Throughout generations, the nutritional and functional impacts of various fermented dairy products have been widely recognized and practiced. However, it was not until around 1910 that scientific research began to investigate into the implications of fermented milk products.

Irgo is a fermented milk product that bears similarities to yogurt. It exhibits a thick, smooth texture with a consistent appearance and typically maintains a white colour when prepared meticulously. This semi-solid product is characterized by a pleasant aroma and flavour. (Admasu & Cione, 2016). These fermented products have different vernacular names such as *Ititu, Irgo, Meomata or Geinto* among the Oromo, Amhara, Wolayta or Sidama people, respectively (Zelalem *et al.,* 2011).

The fermentation process is usually carried out using natural microorganisms, without using defined starter cultures to initiate fermentation process, particularly in traditional dairy practices where fermentation proceeds without active regulation. In this case, fresh milk is typically left at room temperature or in a moderately warm environment to encourage fermentation (Andualemm *et al.,* 2014).

Traditional dairy products of Ethiopia are mainly fermentation products of lactic acid bacteria (LAB). *Irgo* is a popular traditional fermented milk product of Ethiopia, fermented milk products fulfilling multiple purposes in the rural life like consumed as food and as beverages (Tesfemariam *et al.,* 2017). *Irgo* is considered as a special food which serves as a basis for further processing and it is particularly used as a nutritional support to sick people, children and to pregnant and lactating mothers. It is an important part of the diet because it is rich in major nutrients protein, carbohydrate, vitamins, fat and, when consumed fresh, Constitutes low health risks due to its low pH, at which most pathogenic and spoilage organisms are inhibited.

Irgo is also produced in urban areas but in this case traditional utensils are substituted by glasses or cans and smoking of utensils is not practiced. In contrast to traditionally prepared *Irgo* with 15–20 days shelf life, the urban product may be stored without spoilage only for relatively short periods unless refrigerated. The relatively low pH of *Irgo*, ranging from 4.3 to 4.5, enables its further storage. Unhygienic milk production and processing and absence of starter cultures result in *Irgo* with variable characteristics and short shelf life and higher spoilage risks due to the wide variety of microorganisms in the product. If the temperature during incubation is too high, fermentation is rapid and over-souring occurs, causing a separation of the liquid and solid phase and gas production, thus leading to deterioration of appearance and texture (Gonfa *et al.,* 2001).

2.2. Handling Practices of Milk and *Irgo*

Proper handling practices are essential to maintain the quality, safety, and shelf life of yogurt. Milk and milk products are major food for human all over the world. Milk is virtually sterile when it is synthesized in a healthy cow's udder. However, as soon as it leaves from the heat becomes contaminated with microorganism and spoiled till consumption or further processing Tolessa (2016). It is an ideal medium for microorganisms and as it is a liquid and nutritious, it is very easily contaminated and invaded by bacteria. As a result, hygienic milk handling practices should take into account such as the sanitation of milking environment, the hygiene of the milkier and utensils used to collect and store milk (Tsedey and Asrat, 2015).

In Ethiopia, milk hygiene handling practice is below the standard due to insufficient pre milking handling practices like washing udder with clean water, cleaning milking barn, drying the udder with individual towel. In addition, washing of milkers' hands and milking utensils, using of poor quality and non-boiled water for cleaning of udder and insufficient post handling practice like poor hygiene of 6 milk equipment and storage containers, prolonged storage, transportation and retailing practices predispose the milk to microbial contamination (Tsedey and Asrat, 2015; Fufa *et al.*2019).

Moreover, use of plastic containers for handling and transporting of milk increase the risk of contamination of milk higher, since as the number of plastic containers increased the chance

of contamination is also increased and most plastic containers have characteristics that make them unsuitable for milk handling (Tsedey and Asrat, 2015). Furthermore, using clay pot for storing milk is another factor. This device for producers is inconvenient for hygienic cleaning, it also harbors bacteria, which causes milk spoilage and consequently imposes risk of quality deterioration (Tsedey and Asrat, 2015).

The dairy producers clean their milking utensils in different ways, for instance, washing with or without hot water followed by smoking with different aroma producing plants like *Woira* (Oleaafrican), and Tid (Juniperousprocera) used for flavoring and extending the shelf life, since fumigation have the power of disinfecting (sterilizing) the milking equipment. Thus, reducing the numbers of microorganisms and thereby extending the shelf life of milk products, and thereby reducing spoilage (Tsedey and Asrat, 2015). Shewangzaw *et al.* (2016) reported that about 55.6% of the producer should not used refrigerator for handling of milk and its products.

This may favor the multiplication of bacteria and spoilage of milk and milk products. Generally, poor handling practices result in higher the bacterial count, which in turnmay cause spoilage of the milk and poor yields of its products. Moreover, the rise of bacterial count is unsafe since it cause food borne diseases and imposes a great health risk on the consumers Tolosa (2016).

2.3. Physicochemical Properties of *Irgo*

2.3.1. Protein

Proteins in yogurt are of excellent biological quality, as are that in milk, because the nutritional value of milk proteins is well preserved during the fermentation process (Gursoy *et al*., 2010). It has been argued that protein from yogurt is more easily digested than is protein from milk, as bacterial pre digestion of milk proteins in yogurt may occur (FAO, 2013). Both the caseins and the whey proteins in yogurt are rich source of amino acids (93%) and high in nitrogen availability. Amino acids like proline and glycine are present in free form and higher contents in yogurt than in milk (Damian, 2013).

The protein content of milk can be increased by concentration of milk or by dry-matter enrichment. Proteins in yogurt are of excellent biological quality, as are that in milk, because the nutritional value of milk proteins is well preserved during the fermentation process. Therefore, yogurt is recommended for sick and convalescent people (Ebringer *et al*., 2008). On average, yogurt usually contains around 8-10 grams of protein per 6-ounce serving, but this can vary depending on the specific brand and type of yogurt. Greek yogurt, for example, is typically higher in protein compared to regular yogurt due to the straining process that removes more whey (Sodini *et al*., 2004).

2.3.2. Fat

Although much of the yogurt sold in industrialized countries is produced from skimmed milk, traditional yogurt has always contained some 3-4 g per100 g milk fat; indeed concentrated yogurt will contain 9-10 g per100 g fat (Tamine and Robinsion1999). Yogurt may contain fat levels ranging from zero to 10%, with typical values falling between 0.5% and 3.5% (Lucey and Sigh, 1998). The fat content in yogurt is often regulated by national quality standards.

Legal guidelines or proposed regulations regarding the chemical composition of yogurt in different countries often categorize yogurt into three types based on fat content (full, medium, and low) (FAO/WHO, 1973). This classification system aids in setting compositional standards for yogurt, ensuring product consistency and consumer protection. The influence of fat content on yogurt texture is closely tied to the homogenization process, commonly employed in yogurt production. Fat contributes to the perceived creaminess and enhances the mouth feel of yogurt products (Sodini *et al.,* 2004).

Conjugated linoleic acid, a type of essential fatty acid found almost exclusively in the fat of dairy products, can be obtained only through the diet because it is not produced by the human body (Miller *et al*., 2006). CLA has been shown to be a powerful natural anti-carcinogen that also can reduce the risk for cardiovascular disease, help fight inflammation, reduce body fat, especially abdominal fat, lower cholesterol and triglycerides, increase metabolism, lower insulin resistance and enhance the immune system (Lee and Lucey , 2010).

2.3.3. Moisture

The moisture content of yogurt refers to the amount of water present in the product. The moisture content of yogurt typically ranges from 75 to 85%, depending on factors such as the type of milk used, the processing method, and any added ingredients. Moisture plays a critical role in the texture, consistency, and mouth feel of yogurt. Adequate moisture content is necessary to achieve a creamy and smooth texture in yogurt products. Monitoring and controlling moisture levels in yogurt production are essential to ensure product quality, shelf life, and consumer satisfaction (Lucey & Singh, 1998).

2.3.4. Total solid

To comply with regulatory standards and ensure consistent product quality, it is crucial to standardize the total solids content, primarily the milk protein content, and milk fat content. The total solids level in milk plays a vital role in both the texture and aroma of yogurt produced (Tamime and Deeth, 1980). For yogurt production, the total solids content in milk can range from as low as 9% in skim yogurt to over 20% in other type of yogurt. As highlighted by Tamime and Robinson (1999), enhancing the milk total solids content, particularly the protein content, from 12 to 20% leads to improved consistency in yogurt products.

The optimum yoghurt consistency could be achieved from milk containing 14-16% total solids. Although small difference in consistency is achieved when the content of total solids varies from 16–20 %, there is little interest in the use of concentrations above 16 % and, most low-fat yoghurt fall within the range of 14-15% (Penna *et al*. 2006). In general, total solid level in excess of 25% is not recommended as it adversely affects the availability of moisture and hinders starter culture activity.

2.3.5. Ash

Ash content in yogurt refers to the mineral content left behind after the organic components have been burned off. The ash content in yogurt is typically low, ranging from about 0.5% to 0.8%. This ash content primarily consists of minerals such as calcium, phosphorus, potassium, and magnesium, which are essential nutrients for the human body. The mineral composition of yogurt can vary depending on the type of milk used and any added ingredients. Monitoring the ash content in yogurt is important for assessing its nutritional

value, particularly in terms of mineral content, and ensuring that it meets regulatory standards for food quality and safety (Lucey & Singh 1998).

2.3.6. Titratable acidity

The titratable acidity of yogurt is a measure of its acidity level, reflecting the total amount of acid present in the product. It is often expressed as the amount of lactic acid in grams per 100 milliliters of yogurt (g/100 ml). The titratable acidity of yogurt typically ranges from 0.8 to 1.2%, although values can vary based on factors such as the type of milk used, the fermentation process, and the specific bacterial cultures employed. This acidity is primarily a result of lactic acid production by lactic acid bacteria during fermentation, contributing to the characteristic tangy flavor and texture of yogurt. Titratable acidity is an important parameter in evaluating the quality, taste, and shelf life of yogurt products (FAO/WHO, 1973).

2.3.7. pH

The pH of yogurt, typically ranging from 4.0 to 4.6, is a crucial factor that influences its taste, texture, and shelf life. This acidic pH level is primarily a result of lactic acid fermentation by specific bacterial strains during the yogurt-making process. Lactic acid bacteria, such as *Lactobacillus bulgaricus and Streptococcus thermophilus*, convert lactose in milk into lactic acid, lowering the pH.

The acidity not only gives yogurt its characteristic tangy flavor but also helps preserve the product by inhibiting the growth of harmful bacteria. pH levels can vary slightly based on factors like fermentation time, temperature, and the specific bacterial cultures used, impacting the overall quality and stability of the yogurt product (Tamine and Robinsion,2007).

2.3.8. Carbohydrate

In the case of natural yogurt, a number of mono and disaccharides are present in trace amounts, but lactose remains the dominant sugar in natural yogurt; even after fermentation, the product may contain some 4-5 g per 100 g lactose. The reason for this residue is that the processed milk is often fortified to 14-16 g per 100 g total solids (i.e. up to about 8 g per 100 g lactose), so that the lactose content of the end product is little different from normal milk

(Tamine and Robinsion, 2000). What is different, however, is the effect that these apparently identical levels of lactose can have on people who are so called lactose intolerant or lactose maldigestors and the nature of this reaction is of considerable medical interest (Damian, 2013).

2.4. Microbial Quality of *Irgo*

The dairy industry manufactures a wide range of milk products, which are considered perishable. Microbial contamination of dairy products including yoghurt may occur from various sources including utensils, environment, handler, additives, and packaging materials. A large number of microbes, which cause spoilage, are isolated from yoghurt (Pal, 2013).The changes in the physical, chemical and microbiological structures of yoghurt determine the storage and shelf life of the product (Sofu and Ekinci, 2007).

The use of poor quality of milk, unsuitable starter culture, improperly cleaned utensils, and unfavorable temperature of incubation, are responsible to lower the quality of yoghurt. Yoghurt should not be free zed, as it affects the texture and quality. Further, it should be protected from other foods with strong odor by sealing it tightly. Yoghurt should be kept in refrigerator after it is purchased. Clean spoon should be used to take yoghurt into the bowel. In order to avoid contamination, it is imperative not to return unused portion of yoghurt to the original container (De, 1980).

By cleaning, 10% of the germs that are present in milk eliminate roughly. Most of the time, while cleaning and disinfection, not all bacteria are eliminated and killed. All bacterial and fungal counts are expressed as colony forming units per milliliter or per gram for liquid and solid foods, respectively (Adebayo *et al.*, 2014). The commonly used microbial quality tests for milk and milk products include determination of total bacterial count (TBC) and coliform count (CC) and yeast and mould counts, *Enterobacteriaceae*, etc. (Alganesh, 2016; Fufa *et al*., 2019).

2.4.1. Total bacteria count

The Total Bacteria Count (TBC) in yogurt refers to the total number of viable bacteria present in the product. This count includes all types of bacteria, both beneficial (such as lactic acid bacteria used in fermentation) and potentially harmful bacteria. The TBC is an

important parameter in assessing the microbiological quality and safety of yogurt (Huis, 1996). In general, the total bacteria count in yogurt should be controlled and maintained at appropriate levels to ensure product quality and safety. High levels of total bacteria count may indicate poor hygiene during production, improper storage conditions, or post-processing contamination (Habtamu *et al.,* 2018). The acceptable level of Total Plate Count (TPC) in yogurt typically ranges from 10,000 to 100,000 colony-forming units per gram (CFU/g) depending on various factors such as regulatory standards, industry practices, and specific guidelines (Hui, 2004).

2.4.2. Coliform Count in *Irgo*

Coliforms are responsible for the development of objectionable taints in milk and milk products rendering them of inferior quality or even unmarketable (Mituniewicz-Małek *et al.,* 2017). The coliform count in yogurt is a measure of the presence of coliform bacteria in the product. Coliform bacteria are a group of bacteria that are commonly used as indicators of sanitation and the potential presence of harmful pathogens in food products. In the context of yogurt production, coliform counts are used as a measure of hygiene and food safety (Agu K.C. *et al.,* 2014).

It covers all gram-negative, non-spore forming, aerobic and facultative anaerobic rods capable of fermenting lactose with the formation of acid and gas at 35 ^{o}C within 48 hours. The majority of them are members of the genera *Klebsiella, Enterobacter,* and *Escherichia coli*. The presence of coliform bacteria in yoghurt is a sign of unhygienic manufacturing, processing, or storage conditions. So, the fact that they are present in such significant quantities in dairy products is a sign that they may be harmful to consumers health (Abel *et al.,* 2016).

Yogurt is typically not expected to contain high levels of coliform bacteria. Elevated coliform counts in yogurt could indicate issues with sanitation during production or contamination post-processing. Regulatory standards often dictate the acceptable levels of coliform bacteria in yogurt and other dairy products to ensure consumer safety. The presence of coliforms indicates a safety risk, and the numbers should therefore be of the minimum recommended levels in milk products (Alganesh, 2016; Fufa *et al.,* 2019).

Monitoring and controlling coliform counts in yogurt production are essential to maintain product quality and safety standards. This involves implementing good manufacturing practices, proper sanitation procedures, and regular testing to ensure that coliform levels remain within acceptable limits (El- Bakri *et al.,* 2009).

2.4.3. Yeast and mould count

Yeasts & molds are a major cause of spoilage of yoghurts in which the low pH provides a selective environment for their growth. Yeasts and molds commonly associated with milk and milk products are *Saccharomyces* spp., *Penicillium spp., Rhizophus spp., Aspergillus spp., Geotrichum Candidum, Alternaria spp., and Cladosporium spp*. (Vishweshwar and Krishnaiah, 2005).

The mould and yeasts are the common spoilage organisms of fermented foods (Filtenborg *et al*., 1996; Robinson,2000).The presence of *Aspergillus and Penicillium* in yoghurt should be viewed seriously as both the fungi produce mycotoxins, which are deleterious on human health (Pitt and Hocking,1999; Pal, 2002).

Yoghurt produced under good manufacturing practice (GMP) should not contain > than 10 yeast cells, and should have a shelf life of 3-4 weeks at 5 0c. Yoghurt having initial yeast counts of >100 CFU/g tend to spoil quickly (El- Bakri *et al.*,2009). Hygienic practices in the production can improve the microbial quality standards of yoghurt. Strict supervision and stringent quality control standards are imperative to improve the microbial safety of the product, and ultimately reduce the microbial hazards.

2.4.4. Escherichia coli (*E.coli*)

Escherichia coli is one of the most prolific microorganisms in human intestinal tract and it is normally harmless; however, certain strains can be pathogenic, like *enterohemorrhagic E. coli* (EHEC). These strains carry genetic determinants for attaching-effacing lesions that cause hemorrhagic colitis with severe abdominal pain and cramps followed by bloody diarrhea. In addition, the Shiga-like toxin production leads to additional intestinal diseases, such as hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Willey *et al.*, 2008).

A major example of EHEC is *E. coli* O157:H7, which was first recognized as a human pathogen Riley *et al.*,1982) when it was associated with two outbreaks of hemorrhagic colitis. Since then, many other foodborne outbreaks have been reported involving different products such as yogurt (Morgan *et al*,.1993). Although yogurt is considered safe due to low acidity, some authors have reported *E. coli* O157:H7 survival during the storage period (Bachrouri *et al*., 2002; Evrendilek *et al*., 2007). It has been shown that *E coli* O157:H7 cells have an effective mechanism to resist to extreme acid stress situations, and its resistance depends on the interaction with environmental compounds (Meng *et al*., 2007).

The presence of *Escherichia coli (E. coli)* in yogurt is a serious concern as it is an indicator of fecal contamination and potential pathogenicity. *E. coli* is a type of bacteria commonly found in the intestines of humans and animals. While most strains of *E. coli* are harmless, certain pathogenic strains can cause foodborne illnesses and pose health risks if consumed. In yogurt production, the presence of *E. coli* is not expected or acceptable. Manufacturers follow strict hygiene practices and quality control measures to prevent contamination of yogurt with harmful bacteria like *E. coli*.

Regulatory standards typically set limits on the presence of *E. coli* and other pathogens in dairy products to ensure consumer safety (Mattar *et al*., 2012). If *E. coli* is detected in yogurt, it indicates a serious food safety issue and potential health risk. Contaminated batches of yogurt should be promptly removed from the market, and corrective actions must be taken to identify and address the source of contamination to prevent future occurrences. Regular testing for pathogens like *E. coli* is crucial in the dairy industry to uphold food safety standards and protect public health (Mattar *et al*., 2012).

2.4.5. Microbiological specifications of yoghurt

A microbiological criterion defines the limit above, which the food is considered to be contaminated at an unacceptable level with a microbe, its toxin or metabolite, and therefore, considered to be unsafe for human consumption. Undesirable microbes constitute the primary hazard to the safety, quality, and wholesomeness of milk and milk products. In order to maintain the safety of the dairy products, microbiological guidelines is an essential requirement (Pal, 2013). Food Safety and Standards Authority of India established under the

Food Safety and Standard Act 2006 is the regulatory body related to food safety and laying down of the standards of food in India. The microbiological criteria of yoghurt under Indian regulations is presented in Table 1.

Table 1: Microbiological standard of yoghurt

Requirement	**Yoghurt**
Total plate count	< 10, 00, 000 cfu/g
Coliforms	< 10 cfu/g
Anaerobic spore count	0 cfu/g
Escherichia coli	0 cfu/g
Listeria monocytogenes	0 cfu/g
Salmonella	0 cfu/25g
Shigella	0 cfu/25g
Staphyloccus aureus	< 100 cfu/g

Source: Pal (2013)

2.5. Quality and Safety of a Fermented Product

The quality and safety of a fermented product entirely depend on the spices and spices composition of LAB that are involved in the fermentation process. In the time course of ergo fermentation microorganisms such as *Streptococcus, Lactobacillus, Lactococcus and Leuconostoc* are carried out the souring process. They also detected fairly high numbers of micrococci spore formers and coli forms during the first 14 to 16 hours of fermentation.

The lactococci spices are the most dominant one throughout the fermentation that reached colony counts as high as 10 cfu/ml at the end of the fermentation process. At the same time, the aerobic mesophilic bacteria have also shown similar colony counts and the yeast population was increased up to 10 cfu/ml at 24 hours. At the end of fermentation, pH value highly reduced and titratable acidity of the fermented product was 0.75%.

The type of microorganisms involved during ergo fermentation of raw milk Initial aerobic mesophilic counts varied between 10 and 10 cfu/ml among the various fermenting milk. Normally ergo was formed after 24 hours and the average aerobic mesophilic count was

greater than 10 cfu/ml. Coliform counts, which were less than 10 - 10 cfu/ml at 4 initiation of fermentation, reached 10 CFU/ml within 12 hours but this count decreased markedly thereafter. LAB had initial counts of <10 - 10 cfu/ml and maximum counts (10 - 10 cfu/ml) were attained at 24 hours. Initially, 79 yeasts were below detectable levels but gradually reached colony counts of 10 cfu/ml at 24 hours. The pH value of 5 most samples was reduced gradually up to 12 hours and reduced sharply thereafter up to values of 4.3 or below. Average initial titratable acidity of 0.16% was increased to complete 0.88% at ergo formation.

To study the effect of container smoking on the microbiological and biochemical qualities of fermenting *Irgo*, raw milk was allowed to sour naturally at ambient temperatures (25-30°C) in smoked or non-smoked containers Milk in smoked containers had a lower rate of pH drop and the fermented product had good flavor for a longer time after coagulation.

The total count of non-lactic acid bacteria in milk in non-smoked containers reached a high count (greater than 10 cfu/ml) within 12 hours, whereas milk in smoked containers required more than 24 hours reaching this level. Similarly, the growth of coli forms and lactic acid bacteria was slow in milk in smoked containers, thus assuring good and slow development of flavor components, safety of finished product and better keeping quality. *Lactobacilli* dominated the flora of the fermented product in non- smoked containers, while *lactococci* were not equally dominant in fermented milk in smoked containers.

All the test pathogens could grow to levels as high as 10 - 10 cfu/ml within 12 hours in fermenting milk. Smoking of containers significantly retarded the growth of the test pathogens, but only until 12 hours. Growth of LAB in souring milk resulted in inhibition of *Salmonella typhimurium and Salmonella enteritidis* between 48 and 60 hours of fermentation of milk in non-smoked and smoked containers. In most cases, household preparation of *Irgo* requires one-day incubation at ambient temperatures. The milk coagulates within 24 hours and *Irgo* is usually consumed preferably at this time due to its good flavor. Bacteria used, and the manufacturing process are the main factors that affect the physical properties of yogurt, such as its colour, flavour, aroma, and texture.

2.6. Sensory Evaluation

Sensory evaluation is a scientific discipline used to suggest measure, analyses and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing (Stone, 2004). For the food industry, the detection of oxidative off-flavor's by taste or smell is the main method of deciding when a lipid containing food is no longer fit for consumption.

Many of the lipid oxidation products have distinctive flavor characteristics that contribute to the overall perception of objectionable changes in an oxidized food. However, depending on the type of food and the stage and conditions of oxidation, such off-flavors vary not only in their intensity but also in their character. Terms such as rancid, stale, beany, metallic, cardboard, green, fishy, etc., are often used to describe the state of lipid oxidation in foods.

The testing of oxidative off-flavors can be conducted by trained taste panels using special score forms. The reproducibility of sensory analysis is generally considered worse than that of chemical or instrumental methods. More recently, use of an electronic nose to monitor the formation of volatile compounds associated with off-flavors from lipid oxidation has been proposed to supplement information from human sensory panels (Shahidi, 2005).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in the selected areas of the central highlands of Ethiopia namely Holeta and Addis Alem town.Holeta is located in the Oromia National Regional State, West Shewa zone, at a distance of 29 km far away from Addis Ababa. It is bordered by Olonkomi in the west and southwest and Menagesha in the northern Holeta was situated at latitude of 9° 00'N and, 38° 30'E longitude. The town is situated at an altitude of 2400 m above sea level. Holeta experience a bimodal with the main rainy season from June to September and a short rainy season from March to May. The average annual rainfall is 1144 mm with the average annual temperature of 6 ^{0}C and a maximum temperature of 22 ^{0}C. Addis Alem is located also in West Shewa Zone of the Oromia region, west of Addis Ababa, central Ethiopia. The total driving distance from Addis Ababa to Addis Alem is 45 kilometers. This town has latitude and longitude of 9°2′N 38°24′E with an elevation of about 2,360 meters above sea level. Adis Alem typically receives about 93.83 millimeters (3.69 inches) of precipitation and has 199.44 rainy days (54.64% of the time) annually. The town has the minimum annual temperature of 9 ^{0}C and a maximum temperature of 20 ^{0}C (Mekibib *et al.*, 2010).

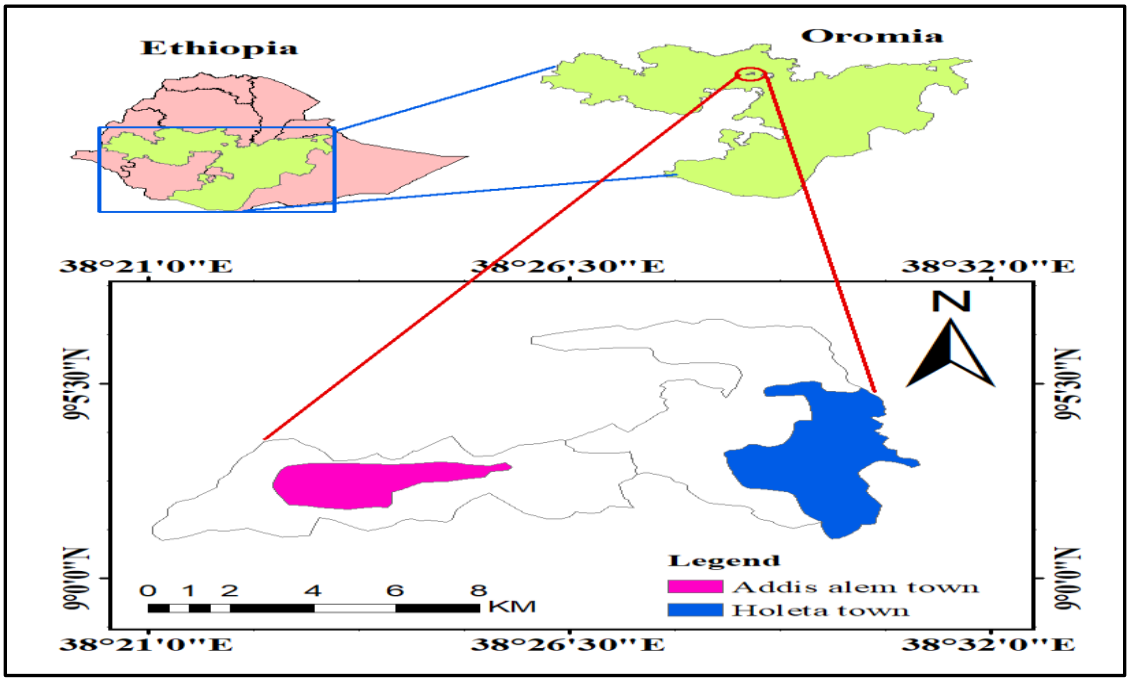


Figure 3.1 Location maps of study area

3.2. Study Design

A cross-sectional research design combined with laboratory analysis was employed in this study. Initially, the survey data capturing questionnaire was developed to gather the required information from the *Irgo* sellers. Then *Irgo* samples were collected from them for laboratory analysis to evaluate the physicochemical and microbial quality.

3.3. Sampling Techniques and Sample Size Determination

In order to collect the data on hygienic practices of *ergo* production a semi-structured questionnaires was prepared. A purposive sampling technique was used to select sellers. During the study time, *Irgo* sellers having shop in sampled towns of the study area were 100. The sample size was estimated as described by Yamane's or Slovin's (1967) using the following formula:

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

Where N = population size, n = sample size, and e = precision level at 95% confidence interval then e = 0.05, Based on the above formula, the sample size (n) was calculated as follows.

n= $100/1+100(0.05)^2$ =80

In that aspect, 80 *Irgo* sellers were selected purposively; based on availability of *Irgo* during the time of sample collection and willingness to participate in the research. For interviewing, 80 *Irgo* sellers (40 from Holeta and 40 from Addis Alem) were selected based on traditional *Irgo* production purposively to asses the hygienic practices during *Irgo* production.

3.4. Data Collection

Data was collected by laboratory and observational questionnaire based results. In order to collect the necessary data on hygienic practices of *Irgo* production practices a semi-structured questionnaire was used. The assessment was concentrated on hand washing, cleaning frequency of shop, cleaning of *Irgo* equipment, sources of water and treatment, detergent used for washing of utensil.

From previously surveyed seller 30 *Irgo* samples (15 from Holeta and 15 from Addis Alem) were randomly collected for laboratory analysis. Each of 500 ml of *Irgo* samples was collected for microbial and physicochemical analysis from the study areas. To avoid contamination

during sample collection, a maximum sterile working condition was implemented. The sampl es were collected in a sterile cup bottle and labeled used for laboratory analysis. The all *Irgo* samples were transported to the dairy microbiology laboratory of Holeta Agricultural Research Center and immediately after sampling on icebox containing ice pack, where the laboratory analysis was carried out.

3.4.1. Analysis of physicochemical composition of *irgo*

3.4.1.1. Determination of protein content

The total protein content was determined using the formaldehyde titration method. Ten ml of *Irgo* will be added into a beaker. Then it was mixed with 0.5 ml of a 0.5% phenolphthalein indicator and 0.4 ml of a 0.4 % potassium oxalate. The sample was then be titrated with 0.1N Sodium Hydroxide solution using a digital dispenser/burette. Until the pink hue becomes vivid, the titration will be continued (Sanett Matela *et al.,* 2019). Finally, the burette reading was then being recorded. The reading was multiplied by a factor 1.74 (Sanett Matela *et al.,* 2019).

3.4.1.2. Determination of fat

The fat content was evaluated using the association of official analytical chemists (AOAC), 2000 method. For 30 minutes, samples were stored in a water bath at 37°C to be warmed to the cow's normal body temperature. Pipetting ten millilitres of powerful sulphuric acid into a butyrometer was take place. 11 ml of *Irgo* samples were added using milk pipette into a butyrometer bearing the sulphuric acid and then one ml of amyl alcohol was added. Once all samples have been broken down by the acid, the butyrometer stopper was applied, and the sample was shaken and inverted many times. The butyrometer was then spending five minutes in a 65°C water bath. The sample was placed in a Gerber centrifuge to four minutes at 1100 rpm (rotations per minute). Finally, the sample was placed in a water bath set at 65 ^{o}

C for 5 minutes while the butyrometer reads the sample's fat percentage (Sanett Matela *et al.*, 2019).

3.4.1.3. Determination of moisture content

The percentage of moisture content was determined by oven method. Briefly, 2g of *Irgo* samples were dried in the oven for 24 hours at 100^0C. The percentage moisture content was calculated by the following formula:

$$\% \text{ moisture} = \frac{W1-W2}{W1}\ x\ 100$$

Where, W_1=initial weight of sample; W_2=weight of the dried sample.

3.4.1.4. Determination of total solid

To determine the total solid, three grams of *Irgo* sample was pipette into two crucibles that has already been pre-weighed and dried. The crucibles were immersed in a kettle of boiling water for 30 minutes. The oven was drying the samples for 24 hours at 102°$\pm$ 2℃. The dried samples were then taken out of the oven, weighed, and placed in a desiccators after cooling. The samples were going through a second oven drying process that was takes an hour. Reweigh after chilling.

The drying procedure was repeated until the difference in weight between two successive weighing was not more than 1 mg (Yadav & Shukla, 2014) . Then the total solid was calculated as follows:

$$\text{Total solid} = \frac{M_2-M_0}{M_1}X100$$

Where: M_0 = the mass in grams of the crucible

M_1 = the mass in grams of the test portion

M_2 = the mass in grams of dried crucible and test portion

3.4.1.5. Determination of ash content

The total ash content was determined gravimetrically by igniting the dried *Irgo* samples used for total solids determination in a muffle furnace in which the temperature was slowly raised

to 550^{o}C until five hour(Ojuko & Tadese, 2018). The sample was ignited until carbon (black colour) disappears or until the ash residue becomes white. Finally, the ash was removed from the furnace and cooled in desiccators. The ash content was calculated as:

$$\text{Ash (\%)} = \frac{Burnt\ crucible + sample\ weight - oven\ dry\ crucible\ weight}{Sample\ weight} \text{ x } 100$$

3.4.1.6. Titratable acidity (TA)

The titratable acidity of *Irgo* sample was tested by following the standard methods of AOAC, 2005. *Irgo* samples of 10ml were added into a 100ml white crucible and 3-4 drops of 0.5% phenolphthalein indicators was added and stayed for two minutes. After two minutes, it was titrated with standard 0.1N sodium hydroxide until pink color persists for 10 seconds. The titratable of the sample was calculated as:

$$\text{Titratable acidity} = \frac{ml\ of\ 0.1N\ NaOH\ x\ 0.009\ ml}{ml\ of\ the\ sample} \text{ x } 100$$

3.4.1.7. Determination of pH

The pH measurement was carried out using a digital pH meter calibrated with pH 4 and 7 buffers (AOAC,2000). 25 ml of *Irgo* sample was transferred to a 50 ml beaker. The pH probe was inserted into the sample, and the beaker was gently swirled until the pH reading stabilized before noting the value.

3.4.1.8. Determination of carbohydrate

Total carbohydrate content of each *Irgo* sample was determined by nitrogen free extractives and carbohydrate difference using the method described by Bryant et al., 1988. These were done by subtracting the total percentage values of moisture, ash, protein, fat, crude fiber obtained from 100 %. Thus, it was determined using a mathematical function below as described.

$$\text{\% Carbohydrate} = 100\% - (\%moisture + \%ash + \%protein + \%fat + \%crude\ fiber)$$

3.4.2. Microbial quality analysis

For the microbiological analysis of *Irgo* samples, Total Bacteria Count (TBC), Coliform Count (CC) as well as Yeast and Mold count (YMC) was considered. For these counts, peptone water was sterilized by autoclaving at 121^0C for 15 minutes. Similarly, plate count agar was used for the determination of total bacteria count sterilized by autoclaving at 121^0C for 15 minutes. Potato dextrose agar was used for determination of yeast and mould count sterilized by Autoclaving at 121^0C for 15 minutes. Moreover, violet red bile agar was used for the determination of coliform count also sterilized by boiling for about 2 minutes until it was completely dissolved (Richardson, 1985). For all tests, the media used was prepared according to the directions given by the manufacturers.

Irgo samples used for microbial analysis were put aseptically in sterile bottles. For total bacteria count (TBC), dilutions was selected so that the total numbers of colonies grown on a plate was between 30 and 300, while for CC dilutions that resulted counts between 15 and 150 per plate was selected. The estimated number of colonies (colony forming units) per gram of *Irgo* sample was calculated according to (Silva *et al.* (2018) as described below:

$$\text{Ncount} = \frac{C}{Vxd}$$

Where:

N = Number of cfu/g or ml of test sample

C = the number of colonies on the counted plate

d = the dilution rate of the counted plate

v =the inoculated volume of this dilution

3.4.2.1. Total bacteria count

The sample was homogenized and serially diluted in 0.1% peptone water (25g of *Irgo* sample in 225ml of 0.1% peptone water for initial dilution and by transferring 1ml of the previous dilution in 9ml of 0.1% peptone water). After through mixing using a vortex mixer, serial dilutions was prepared under a horizontal laminar flow cabinet from 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. One ml of the sample was placed on sterile petri dish and pour plated with 10- 15ml molten plate count agar (45^0C) and allowed to solidify for 15 minutes and incubated for 48

hours at 37℃. Finally, the counts were made using a colony counter. Plates with countable colonies between 30 to 300 cfu/plates was selected to calculate cfu/g (Richardson, 1985).

3.4.2.2. Coliform bacteria count

After overtaking the *Irgo* sample portion, 25g of *Irgo* sample was diluted in 225ml 0.1% of peptone water for initial dilution. After through mixing using a vortex mixer, serial dilutions was prepared under a horizontal laminar flow cabinet from 10^{-1}, 10^{-2}, 10^{-3}, 10,$^{-4}$,10^{-5} and 10^{-6}. One ml of the sample was placed on sterile petri dish and pour plated with 10-15 ml dissolved violet red-bile agar (45^0C) and allowed to solidify for 15 minutes. Plates were incubated at 32℃ for 24 hours. Plates were selected for counts between 15 and 150 colonies for determining cfu/g (Richardson, 1985). Counts were made on typical dark red colonies normally measuring at least 0.5 mm in diameter on uncrowned plates. This was followed by a confirmatory test by transferring and incubating five typical colonies from each plate into tubes of 2% brilliant green lactose bile broth. Gas production after 24 hours of incubation at 25℃ was considered sufficient evidence for the presence of coliforms (Richardson, 1985).

3.4.2.3. Yeast and mould count

Yeast and mould count was made following the recommended standard procedures. After through mixing using a vortex mixer (Model: VM-300, Taiwan), serial dilutions was prepared under a horizontal laminar flow cabinet from 10-1up to10-6. One ml of *Irgo* sample was placed on sterile petri dish and pour plated with 10-15 ml potato dextrose agar and allowed to solidify for 15 minutes. Plates were incubated at 23℃ for 5 days. The numbers of yeast and mould colonies will be then counted (Richardson, 1985). The entire CFU counts in milliliters of *Irgo* sample were computed by using the following equation.

$$N\left(\frac{CFU}{mL}\right) = \frac{\sum C}{[(1xnl)+(0.1xn2)]*d}$$

Where:

N = number of colonies per milliliter of *Irgo*

Σ C = sum of colonies counted on all positive plates.

n_1 = number of plates retained at the lower dilution.

n_2 = number of plates retained in the next higher dilution.

d = the lowest dilution factor from which the first counts are obtained.

3.4.3. Sensory evaluation

The sensory properties of samples were evaluated by 15 evaluators. Ten *Irgo* samples were served in plastic cups and coded. Order of presentation of the samples was randomized. A questionnaire comprising five sensory attributes, namely, appearance, aroma, taste, texture and overall acceptability was given to each of the panelist. A standard 9-point scale was used for the evaluation of sensory characteristics of the samples.

3.5. Statistical Analysis

Collected data were coded and entered in to MS-Excel and it was imported to Statistical Package for Social Science (SPSS) software version 24.0. Descriptive statistics such as mean, standard deviation and percentage were applied to describe the characteristics of the respondents and calculate for physicochemical, microbiological quality and sensory evaluation of *Irgo* samples collected from sellers in the study areas. Least significance difference (LSD) at 5% significance level was used for comparison of means.

4. RESULTS AND DISCUSSION

4.1. Socio-economic Characteristics

The socioeconomic characteristics of respondents are presented (Table 1). A total 80 *Irgo* sellers were involved in the survey from both study areas. The majority of the respondents 72.5% and 77.5% were male in Holeta and Addis Alem, respectively. Most of the respondents (42%) there age were beyond 40 years in Holeta, while 32.5% were between the ages of 36 and 40 in Addis Alem town. This indicates most of them were still in their productive years and coping with the work associated with dairy products. Based on their educational level, the highest numbers of the respondents (35%) were attended basic education and up to grade 4 in both areas and the lowest value were illiterate or had no formal education.

Table 1: Socio economic characteristics of the respondents in the study area

Variable	**Category**	**Study area (%)**	
		Holeta	Addis Alem
Household information	Male	29(72.5%)	31(77.5%)
	Female	11(27.5%)	9(22.5%)
Age	20-25 years	6(15%)	7(17%)
	26-30 years	9(23%)	9(23%)
	36-40 years	8(20%)	13(32.5%)
	>40 years	17(42%)	11(27.5%)
Level of education	Illiterate	2(5%)	5(12.5%)
	Basic education	14(35%)	12(30%)
	Grade 4 (<)	14(35%)	14(35%)
	Grade 5-10	10(25%)	9(22.5%)

4.2. Hand Washing Practices

The hand washing practices in the study areas are presented (Table 2). All *Irgo* sellers reported that they wash their hands before starting the milk processing for *Irgo* preparation. The majority of the *Irgo* sellers (75%) used soap and a towel to clean and dry their hands, while the remaining (25%) did not used soap and a towel to clean and dry their hands in the studied areas. Even though, the higher percentage of the respondents (38.3%) in Holleta town used soap compared to Addis Alem town. Washing, drying and cleaning hands before milk processing is the most important step for maintaining hygiene in *Irgo* production (Yilma, 2010; Bereda *et al* 2013).

Besides, proper hygienic standards of milk during processing and handling are essential to ensure that *Irgo* remains fresh for an extended period (Lencho *et al*., 2018). Bereda *et al.* (2013) and Gurmessa (2015) stated that insufficient hand cleaning can lead to contamination of milk and its products. Using detergent for cleaning is expected to remove milk residues, including microorganisms that affect the microbial quality of milk and its products.

Table 1: Hand washing practices in the study areas

Variables	**Study Area**		**Overall**
	Holeta	Addis Alem	
Hand washing before *Irgo* preparation			
Yes	40(100%)	40(100%)	80(100%)
Use soap and towel to clean and dry hands			
Yes	30(75%)	23(57.5%)	53(66.25%)
No	10(25%)	17(42.5%)	27(33.75%)

4.3. Shop Cleaning Frequency

The shop cleaning frequency in the study areas are presented (Table 3). The majority *Irgo* seller (66.25%) replied that the frequency of a shop cleaning for the selling of *Irgo* is implemented more than one times per week while the only small numbers of *Irgo* seller (15%) did a shop cleaning only once per week. Between both areas, Holeta sellers were cleaning more frequently than Addis Alem sellers in terms of the above variables were. Frequent cleaning of shop ensures profound effect on reducing microbial contamination of processing material (Sintayehu *et al.*, 2008). Clean, dry and comfortable working condition is important to minimize the growth of pathogenic microorganism (Yilma, 2010; Bereda *et al* 2013; Gurmessa, 2015). Thus maintaining the sanitary condition of shop in the study areas was important for the production of good quality *Irgo*.

Table 2: Shop cleaning frequency in the study areas

Variables	**Study Area**		Overall mean
	Holeta	Addis Alem	
Shop cleaning frequency			
Daily	8(20%)	7(12.5%)	15(18.75%)
More than once a week	28(70%)	25(62.5%)	53(66.25%)
Once a week	4(10%)	8(25%)	12(15%)
Total	40(100%)	40(50%)	80(100%)

4.4. Water Sources and Treatment

The water source, treatment and cleaning practices of milk collecting and *Irgo* preparing tools in the study area are presented in (Table 4). As reported by majority sellers (47.5%%) the main source of water is tap water while a small numbers of sellers (18.75%) were reported pond as a sources of water in the studied areas.

The majority of sellers (75%) were treated the water by using heat during the usage of cleaning material. 77.5% of *Irgo* sellers in Addis Alem use treated water while 72.5% were use it in Holeta. All respondents were reported a daily cleaning of the milk collecting and *Irgo* preparing tools. The large number of *Irgo* sellers (57.5%) were treated the milk collecting and *Irgo* preparing tools by washing and smoking with weyira. 60% of *Irgo* sellers in Holeta were treated the milk collecting and *Irgo* preparing tools by washing and smoking with *weyira* but in Holeta they were 55%.

Plants species that are frequently used for smoking milk vessels in the study area were *Olea africana (Ejersa),* and *Premna resinosa* (Tetessa). The main reasons of using these plants were to give good flavor, aroma and increase the shelf life of the milk as well as slow milk fermentation process (Yilma, 2010; Bereda et al 2013; Gurmessa, 2015).

Table 3: Water source, treatment and cleaning practices of milk collecting and Irgo preparing tools

Variable	**Study area**		**Overall**
	Holeta	Addis Alem	
Source of water			
Tap	19(47.5%)	19(47.5%)	38(47.5%)
Well	13(32.5%)	14(35%)	27(33.75%)
Pond	8(20%)	7(17.5%)	15(18.75%)
Heat-treat the water before use			
Yes	29(72.5%)	31(77.5%)	60(75%)
No	11(27.5%)	9(22.5%)	20(25%)
Clean the milk collecting and *Irgo* preparing utensil daily			
Yes	40(100%)	40(100%)	80(100%)
No	-	-	-
Cleaning practice			
Washing only	4(10%)	5(12.5%)	9(11.25%)
Washing and smoking with *weyira*	24(60%)	22(55%)	46(57.5%)
Both	12(30%)	13(32.5%)	25(31.5%)

4.5. Physico-chemical Composition of *Irgo*

The physicochemical quality of *Irgo* collected from Holeta and Addis Alem are presented in table 5. The mean moisture value of Holeta *Irgo* exhibited highest (90.21±2.12), while Addis Alem showed the lowest moisture content (88.26%±1.15). The present study showed higher moisture content of *Irgo* than Ashwini and Rahul (2018) and Eissa *et al* 2010 findings. However, it was agreed with Oumayma *et al*., (2020) and Othman *et al*., 2019 reports.

The higher the moisture, the more the yogurt solution will look watery, whereas if the moisture content is lower, the yogurt will look thick. Referring to the standard value of SNI 2981 (2009), the quality of good yogurt has a moisture value of less than 84%. This is because the high moisture content of yoghurt affects the texture and mouth feel of the product. Therefore, the moisture content of all collected *Irgo* samples from both sites was higher and do not conformed to the moisture content of the standard value.

The results obtained in this study on Table 5 showed that mean fat percentage was lower in Addis Alem town (2.15+1.05) and a little higher in Holeta (2.52+0.65). The lower level of fat might be due to the preparation of *Irgo* from cream extracted milk or the longer the fermentation time. The results were lower than the earlier findings of Eissa *et al* (2010) and Oumayma *et al*., (2020) who reported a fat content of 3.51% -3.99%, 3.60-3.67, respectively. However, it was higher than Ashwini and Rahul (2018) findings.

The guidelines for how yogurt should be composed and labeled suggest that yogurt labeled as 'very low-fat' should have under 0.5% total fat content. Yogurt labeled as 'low fat' should contain at least 0.5% but no more than 2% total fat, and 'full fat' yogurt should have more than 3.2% total fat content (Chandan & Rell, 2006). Therefore, the analyzed results on total fat as presented that the *Irgo* sold in both sites can be better in fat content.

The yoghurt for total solid contents are the measures of non-water components of the *Irgo* samples which includes protein, lactose, mineral and fat. The mean total solids in the study was higher in Addis Alem (11.74+1.15) than Holeta (9.64+1.98) (table 5). These variations in total solid content may be influenced by the composition of milk used. The result was lower than those report of Ashwini and Rahul (2018) and Eissa *et al* 2010.

The percentage of total solids is largely dictated by the legal standards of the country concerned**.** The study by Robinson and Tamime (1975) shows that the recommended range is 14-18%. Kozhev *et al*. (1972) concluded that the best yogurt is made from milk containing 15.5-16.0% total solids. Although the composition of yogurts varies considerably, most low-fat yogurts fall within the range of 14-15%. (Tamime &Robisons, 2007) reported "the total solids concentration level of 24% and above would severely inhibit the growth of *lactobacillus bulgaricus".* On the other hand, the low percentage of total solids in yoghurt could lead to malfunction of starter culture (Adubofour, *et al.* 2014). Therefore, the study result showed that the total solid content of *Irgo* samples in both towns are lower than the above reports.

Protein is a mineral found in milk and yogurt. The higher the protein contained in a product, the product is said to have good nutritional value. The mean protein contents of Holeta and Addis Alem *Irgo* are provided in Table 5. Protein contents of Addis Alem *Irgo* was higher than Holeta's *Irgo.* Differences in protein contents were mostly because of differences in composition of milk processed into yoghurt or dry matter-dependent changes in protein contents.

The obtained result from this study was lower protein content than Eissa *et al* (2010), Seok *et al.,* (2019), Oumayma *et al.,* (2020) findings. In accordance with the Turkish Food Codex standards for yoghurts, which specify a minimum total protein content of 3%, the average protein content from the study areas nearly aligned with these benchmarks.

The pH is the parameter that determines the sample's acidity and alkalinity. The pH range in the current study was 4.24$\pm$0.18 to 4.33$\pm$0.09 for both sites (Table 5). As shown in the table the mean values of *Irgos* were similar to each other. This result is comparable with those reported by Okoye and Animalu (2009); Egwaikhide and Faremi (2010), but lower than those reported by Eissa et al (2010). Granata and Morr (1996) also reported that pH values between 4.0 and 4.4 are considered as necessary for flavor and texture in good-quality yogurts. Similarly, the result obtained in this study agreed with Granata and Morr (1996) and indicating that these *Irgo*s are good quality. The international minimum acceptable standard for pH is 4.4 (FAO, 1979). Inadditoin, based on the normal SNI value, the degree of acidity

at pH generally ranges from 3.8 to 4.5 (SNI 2981:2009). Therefore, the pH values obtained from all samples in the present study was in line with the standards.

The measured titratable acidity in this study as shown in Table 5 was 0.8±0.09 and 0.86±0.06% in Holeta and Addis Alem, respectively. The acidity differences between Holeta and Addis Alem were small, indicating similarities between the sites. The results of the titratable acidity obtained in this study was higher than Gawade (2017) and Seok *et al.,* 2019 that acidity ranged from 0.52 to 0.66 percent in yoghurt. However, it was lower than those of reported by Egwaikhide and Faremi (2010). Titratable acidity approximates the total acidity in a solution. According to TS 1330 Yoghurt Standard, titratable acidity should be between 0.6-1.6 percent. Therefore, acidity of Holeta and Addis Alem *Irgo* were within the specified ranges of TS-1330 and such a case indicated that *Irgo* were fresh.

Table 4: Physico-chemical composition of *Irgo* in the study areas (%)

Parameters	**Holeta (n=15)**	**Addis Alem (n=15)**
Moisture	90.21±2.12	88.26±1.15
Protein	2.64±0.24	2.95±0.71
Fat	2.52±0.65	2.15±1.05
Total solid	9.64±1.98	11.74±1.15
Ash	0.78±0.25	0.97±0.75
Titratable Acid	0.8±0.09	0.86±0.06
pH	4.24±0.18	4.33±0.09
Carbohydrate	3.67±2.11	5.66±1.91

Each value is expressed as mean ± standard deviation.

The ash content represents the total amount of minerals present – such as sodium, potassium, magnesium, calcium, zinc, and iron (Lakshmipathy and Sarada, 2013). The mean ash content of the samples can be seen in Table 5. *Irgo* obtained from Addis Alem relatively had higher ash content than Holeta. The results of ash obtained in this study was higher than Ashwini and Rahul (2018); Othman *et al.*, (2019); Oumayma *et al.*, (2020) that acidity ranged from 0.33 to 0.70 percent in yoghurt.

The mean carbohydrate values of both towns *Irgo* are provided in table 5. This result was lower than the report of Othman *et al.,* 2019. The higher mean value of carbohydrate is for Addis Alem's *Irgo*. The low carbohydrate value is attributed to the process of fermentation, which converts carbohydrate lactose to lactic acid. This makes yoghurt an ideal food for lactose intolerance individuals (Ehirim and Ndimantang, 2004).

4.6. Microbiological Quality of *Irgo*

The microbacteriological quality of *Irgo* was assessed through the collection and analysis of samples from Holeta and Addis Alem. These samples were cultured to determine total Bacterial count (TBC), total coliform count (TCC), total yeast and mold count (TYMC) and *Escherchia coli*.

Total bacterial count (TBC) estimating the total number of microorganisms in *Irgo*. The mean TPC values for each sample source are detailed in Table 6. *Irgo* samples from Holeta and Addis Alem showed mean TPC values of 2.88+1.72log cfu/g and 5.2+ 1.67 log cfu/g, respectively. The findings revealed that *Irgo* from Addis Alem exhibited higher TPC levels compared to that from Holeta. The presence of this microorganism could be due to poor handling during preparation or overgrowth of lactic acid bacteria during fermentation.

During the fermentation of *Irgo* in raw milk sourced from eight dairy farms in Awassa, the average total plate count exceeded 9 log cfu/ml, as documented by Ashenafi (1995). Similarly, the total plate count of *Irgo* showed variability, with counts ranging from around 7.71 log cfu/ml in samples from Sheno to approximately 10 log cfu/ml in samples from Jimma Zelalem's (2010). Besides, Fekadu (1994) observed total plate counts of 8.6 log cfu/ml, exceeding those of 75% of fermented milk samples collected from three villages in Southern Ethiopia.

Additionally, the result of Holeta was lower than the report of Muluken *et al.,* 2016; Gabe, 2023 that total count samples *Irgo* ranged between 3.89 - 4.1log cfu/ml and 9.43±0.17 to 9.49±0.38 log cfu/ml, respectively though Addis Alems' was higher than this report. According to those reports, the current analyzed result showed lower TPC.

Table 5: Microbiological quality of *Irgo* (log10cfu/ml) in the study areas

Microbial group	**Holeta (n=15)**	**Addis Alem (n=15)**
TBC (cfu/g)	2.88±1.72	5.2± 1.67
CC (cfu/g)	3.4± 0.84	3.8±0.59
YMC (cfu/g)	3.37± 0.96	3.57±0.18
Escherchia coli (cfu/g)	Nill	Nill

TPC: Total Bacterial counts, CC: Coliform counts and YMC: Yeast and Mould counts and Nil: means no observable count or no growth.

The results from the current study showed the mean coliform counts for Holeta and Addis Alem were 3.4± 0.84 log cfu/g and 3.8±0.59 log cfu/g, respectively (Table 6). The mean values higher coliform counts observed in Addis Alem *Irgo* samples. Comparing the coliform counts obtained in this study with previous research, it was found that the current study's values were lower. For instance, (Gabe, 2023) reported the mean value of 4.62±0.74 and 4.75±0.45logcfu/g in Sululta Town. An average coliform count of 6.57 log cfu/ml was documented in *Irgo* samples by Zelalem and Faye (2006). Conversely, lower values were observed, with 75% of the samples displaying coliform counts below 4.4 log cfu/ml, as reported by Fekadu (1994). Zelalem (2010) reported an average coliform count of 4.51 log cfu/ml in Ergo samples ranging from Sheno to Jimma.Muluken *et al.* (2016) also observed a lower coliform count of 3.39 to 3.89logcfu/g in traditional yogurt from local market in Addis Ababa, Ethiopia. Based on standards, the coliform bacteria count must not exceed 5 cfu/ml. Presence of coliforms might be from poor hygiene or cross contamination from handlers during milking or production, processing, or storage making *Irgo* (Birollo *et al*., 2001).

As shown in Table 6, mean with standard deviation of yeast and mould count (TYMC) value for each milk sample source has presented. The mean value of yeasts and molds of Holeta and Addis Alem *Irgo* were 3.37± 0.96 and 3.57±0.18 log cfu/g, respectively. The highest quantity of yeasts and molds was found in *Irgo* collected from Addis Alem and lowest quantity was found in *Irgo* of Holeta. The differences in number of yeasts and molds were mainly attributed to production methods, storage conditions and contaminations Gabe, 2023

observed a higher coliform count of 8.18±0.45 to 8.20±0.45 log cfu/g in Sululta town than the current study. Additionally, in a study by Zelalem (2010), the mean yeast and mould counts surpassed 8 log cfu/ml in Ergo samples collected from various regions of the country. Another study by Fekadu (1994) documented yeast and mould counts of up to 4.6 log cfu/ml in fermented milk samples from Southern Ethiopia. The presence of diverse yeast species in milk and its derivatives can lead to product spoilage or, conversely, enhance the flavor of fermented milk due to the ability of different yeast species to utilize various milk components (Gadaga *et al.,* 1999). Moreover, Zelalem *et al*. (2007a) identified the presence of *Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter cloacae, Citrobacter freundii, and Enterobacter sakazakii* in *Irgo* samples obtained from smallholder producers in central Ethiopia.

In TS 1330 Yoghurt Standards, the upper limit for yeast and mold was set as 1.0x102cfu/g. According to Fermented Dairy Products Communique of Turkish Food Codex, number of yeasts and moulds should be maximum $1.0x10^2$ and $1.0x10^3$cfu/g. Based on these values, it was observed that all of the collected *Irgo* from both sites were not complying with TS 1330 Yoghurt Standards and Fermented Dairy Products Communique of Turkish Food Codex. Thus, the result of this study showed that the count of YMC is above the standard. Greater number of yeasts and molds indicate that sufficient care was not taken for hygienic conditions during the production and *Irgo* were not preserved at cold environments. Yeast and mold may generate undesired taste and aroma in yoghurts though high proteolytic and lipolytic activities (Azgın, 1993).

In the present study, *Escherichia coli* viable cells were not detected in both study sites of *Irgo* (Table 6). The obtained result is agreed with Muluken *et al.*, 2016 reports that *E. coli* count in all *Irgo* were not detected. The microbiological analyses indicate that *Irgo* samples were safe for consumption since all counts of microbiological groups were below the standard levels according to the Ethiopian Standards (ES ISO 29981:2012), which states that the acceptable standards of coliform, yeast and mould counts was about 10, the total count was about 50, however, the *E. coli* count was not detected. Hence, the *Escherichia coli* analysis results show either zero presence or non-detection of the bacteria in all *Irgo* samples tested, meeting the established standards.

4.7. Sensory of Evaluation of *Irgo*

The sensory scores are detailed in Table 7, displaying the acceptance of all ten *Irgo* samples from both Holeta and Addis Alem towns by the panelists. Notably, the sample from Holeta town received the highest overall acceptability score of 7.19 ± 0.11. This specific sample also excelled in appearance, aroma, and taste, garnering mean scores of 7.48 ± 0.328, 7.34 ± 0.02, and 6.75 ± 0.02, respectively. In contrast, for texture, the Addis Alem town *Irgo* samples achieved the highest score of 7.65 ± 0.0.

Table 6: Mean and standard deviation (Mean ±SD) for Evaluation of sensory quality of Irgo samples from Holeta and Addis Alem town

Sample	**Parameters**				
	Appearance	***Aroma***	***Texture***	***Taste***	***General Acceptability***
Ho_1	7.41±0.451	7.24±0.02	6.62±0.04	6.45±0.01	6.98±0.135
Ho_2	7.62±0.196	6.89±0.04	6.98±0.02	7.23±0.04	7.18±0.085
Ho_3	7.11±0.054	7.43±0.03	7.04±0.02	6.30±0.01	6.99±0.032
Ho_4	7.45±0.347	7.92±0.01	7.56±0.01	6.56±0.03	7.55±0.1
Ho_5	7.15±0.582	7.16±0.01	7.23±0.02	7.01±0.02	7.24±0.165
Total mean of Holeta's sample	**7.48±0.328**	**7.34±0.02**	**7.12± 0.02**	**6.75±0.02**	**7.19±0.11**
Ad_1	7.2±0.841	6.52±0.02	7.52±0.02	7.06±0.02	6.67±0.236
Ad_2	7.18±0.769	6.69±0.02	7.89±0.01	5.34±0.03	6.88±0.21
Ad_3	7.27±0.718	7.29±0.03	7.62±0.01	6.95±0.05	7.06±0.205
Ad_4	7.44±0.682	7.40±0.01	7.55±0.01	7.01±0.05	7.12±0.190
Ad_5	7.62±0.761	7.35±0.02	7.66±0.02	6.60±0.04	7.18±0.23
Total mean of Addis Alem's sample	**7.35±0.764**	**7.08±0.02**	**7.65±0.01**	**6.62±0.04**	**7.02±0.215**

Values are means ± SD duplicate determinations

Mean values ± standard deviation in each row differ significantly (P<0.05)

Five Yoghurt samples from Holeta (Ho) and Addis Alem(Ad)

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

The study aimed to evaluate the hygiene practices, physicochemical, microbial quality and safety of traditional *Irgo* samples collected from Holeta and Addis Alem town. In the study area there were, practice of washing their hands with detergent before starting to process of milk to preparation of *Irgo* then drying their hands with towel. However, 25% does not used soap and towel to clean and dry their hands in the studied areas.

The majority of the respondents cleaned their shop more than once a week. Still there were sellers who clean once a week. Furthermore, the majority of the respondent cleaned the milk collecting and *Irgo* preparing utensils daily. Although there is a limitation in their cleaning practices. At *Irgo* sellers level the source of contamination was utensil used to collecting, storing and selling of products poor hygienic practices leads to contamination of products.

The chemical analysis revealed that, the total solids (%) and protein (%) in *Irgo* samples were higher in Addis Alem, while fat (%) was lower. The pH and Titration acidity in both Holeta and Addis Alem *Irgo* samples were more than 4 and with the range of 0.70% to 0.93%, respectively. The analysis of *Irgo* samples indicated that the physical and chemical properties aligned with SNI value and Turkish Food Codex standards for yoghurts, except for moisture and total solid, possibly due to the composition of milk used.

Bacteriological differences were evident between Holeta and Addis Alem, reflecting varying hygiene practices. The microbial quality of *Irgo* from the study areas was, falling below accepted standards especially for yeast and mould, even though, the other were between with various findings, indicating inadequate sanitary conditions during *Irgo* preparation and handling. However, *Escherichia coli* meet the standards.

5.2. Recommendations

Based on the present findings the following recommendations are forwarded:

- Strict preventive measures should be adopted to ensure reduced microbes in yoghurt for the good health of all consumers. Therefore, stakeholder authorities should regularly monitor the overall hygienic conditions of dairy products. Regulators to check the problem of poor hygiene and to apply sanctions where necessary must do periodical inspection.
- The *Irgo* producers should educate and follow them on clean and hygienic practices.
- Putting in place useful quality control system is an important tool to bring improvement in product.

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