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Theme

Comparative study between different raw milk (Ewes milk and goats milk) in the Tiaret region

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Dedication

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Dedication

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Dedication

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ملخص

حليب الماعز وحليب النعاج هما منتجان ألبان مهمان يتم استهلاكهما عالمياً. هدفت هذه الدراسة إلى مقارنة الخصائص الفيزيوكيميائية والبكتيريولوجية لحليب الماعز الخام وحليب النعاج في منطقة تيارت.
بدأت الدراسة بنظرة عامة على حليب الماعز وحليب النعاج، حيث تم التركيز على القيمة الغذائية والخصائص الفريدة لهما. فيما بعد، تم إجراء تحاليل فيزيوكيميائية لتقييم معايير مثل محتوى الدهون ومحتوى البروتين ومحتوى اللاكتوز ودرجة الحموضة والكتلة النوعية. كما تم إجراء تحاليل بكتيريولوجية لتحديد وكمية الأحياء الدقيقة الموجودة في عينات الحليب.
كشفت النتائج عن اختلافات معتبرة في التركيب الفيزيوكيميائي لحليب الماعز وحليب النعاج. حليب الماعز أظهر محتوى أعلى من الدهون ومحتوى أقل من اللاكتوز مقارنة بحليب النعاج. فيما يتعلق بالتحاليل البكتيريولوجية، أظهرت كلا أنواع الحليب وجود مجموعات متنوعة من الميكروبات، بما في ذلك البكتيريا اللبنية المفيدة.
علاوة على ذلك، تم تقييم النشاط المضاد للميكروبات لعينات الحليب ضد البكتيريا الممرضة. أظهرت النتائج أن حليب الماعز وحليب النعاج يحتويان على نشاط مضاد للبكتيريا، مما يعيق نمو البكتيريا الممرضة بدرجات متفاوتة.
في الختام، تقدم هذه الدراسة المقارنة نظرة قيمة على الخصائص الفيزيوكيميائية والبكتيريولوجية والمضادة للبكتيريا لحليب الماعز الخام وحليب النعاج في منطقة تيارت. تؤكد النتائج الخصائص الفريدة والفوائد الصحية المحتملة المرتبطة بهذه المنتجات الألبانية. يمكن أن تكون هذه المعلومات مفيدة للمستهلكين ومحترفي صناعة الألبان والباحثين الذين يهتمون بفهم واستخدام خصائص حليب الماعز وحليب النعاج.

الكلمات الرئيسية

حليب النعاج - حليب الماعز - منطقة تيارت - تحليل فيزيوكيميائي - تحليل بكتيريولوجي
جودة الحليب - منتجات الألبان

Abstract

Goat's milk and ewe's milk are two important dairy products consumed globally. This study aimed to compare the physicochemical and bacteriological characteristics of raw goat's milk and ewe's milk in the Tiaret region.

The study began with an overview of goats and ewes milk, highlighting their nutritional value and unique properties. Subsequently, physicochemical analyses were conducted to assess parameters such as fat content, protein content, lactose content, pH, and specific gravity. Bacteriological analyses were also performed to identify and quantify the microbial populations present in the milk samples.

The results revealed significant differences in the physicochemical composition of goat's milk and ewe's milk. Goat's milk exhibited higher fat content and lower lactose content compared to ewe's milk. In terms of bacteriological analyses, both types of milk showed the presence of various microorganisms, including beneficial lactic acid bacteria.

Furthermore, the antimicrobial activity of the milk samples was evaluated against pathogenic bacteria. The findings demonstrated that both goat's milk and ewe's milk possessed antibacterial activity, inhibiting the growth of pathogenic bacteria to varying degrees.

In conclusion, this comparative study provides valuable insights into the physicochemical, bacteriological, and antibacterial properties of raw goat's milk and ewe'smilk in the Tiaret region. The results underscore the unique characteristics and potential health benefits associated with these dairy products. This information can be beneficial for consumers, dairy industry professionals, and researchers interested in understanding and utilizing the qualities of goat's milk and ewe's milk.

Keywords

Ewes milk - Goats milk - Tiaret region - Physicochemical analysis - Microbiological analysis
Milk quality - Dairy products

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List of abbreviation

°D: Dornic degree

Bacillus : *Bacillus cereus*

CO_2 : Carbon dioxide

E. coli : *Escherichia coli*

FAO : Food and Agriculture Organization

H_2: hydrogen

H_2O : Water

H_2O_2 : Hydrogen peroxide

H_2S : hydrogen sulfide

LAB : Lactic Acide Bacteria

M17 : Michel M17.

MH : Mueller-Hinton

MRS : Man Rogosa, Sharpe

NaOH : Hydroxyde of sodium

NIRS:Near-Infrared Spectroscopy

O_2: Oxygen

ONPG : O-Nitrophenyl-β-D-galactopyranoside

P.aeroginosa : *Pseudomonas aeroginosa*

pH: Potentiel Hydrométrique.

S. aureus : *Staphylococcus aureus.*

SNF : Solids-Not-Fat

TSI : Triple Sugar Iron

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Introduction

Introduction

Goat milk and sheep milk are widely consumed and valued for their distinct qualities and nutritional profiles, setting them apart from cow's milk. These milk varieties have been enjoyed by humans for centuries due to their flavorful taste and potential health advantages. In this introduction, we will delve into the essential aspects and attributes of goat milk and sheep milk, emphasizing their composition, nutritional worth, and possible uses **(Benderouich, 2009).**

In recent years, the popularity of goat milk, which is obtained from goats, has soared due to its unique flavor and potential health benefits. Notably, it stands out for its ease of digestion, making it a suitable alternative for individuals with lactose intolerance or those who struggle to digest cow's milk. Goat milk differs in composition from cow's milk, as it contains higher quantities of medium-chain fatty acids like caprylic and capric acids, which are believed to possess antimicrobial properties. Furthermore, goat milk is abundant in essential nutrients such as vitamins A, D, and B12, as well as minerals like calcium, phosphorus, and potassium **(Muehlhoff et *al*., 2013).**

In contrast, sheep milk, sourced from sheep, boasts a distinctive flavor that distinguishes it from both cow and goat milk. Described as rich, creamy, and subtly sweet, sheep milk offers a unique taste experience. It is highly nutritious and renowned for its elevated levels of proteins, fats, and minerals. Notably, sheep milk contains a greater proportion of solids in comparison to cow's milk, contributing to its indulgent and velvety texture. Additionally, sheep milk serves as an excellent source of vitamins, including vitamin A, vitamin E, and vitamin D **(Pulina, 2016).**

Both goat milk and sheep milk have long been employed in diverse culinary customs and the production of dairy goods worldwide. These milk varieties are frequently harnessed for crafting cheese, butter, yogurt, and an array of other dairy-based products, each contributing its unique flavors and textures to these culinary creations. Furthermore, owing to their individual nutritional compositions and potential health advantages, goat milk, and sheep milk are becoming increasingly popular among individuals seeking alternative milk choices **(Park , 2017) ,** so what are the difference in nutritional composition and microbial contamination between goats milk and ewes milk in the tiaret region ?

I. Chapter
Bibliography section

I.1 Nature of milk

For young mammals, including human infants, milk serves as their initial source of nourishment and often remains their exclusive dietary component for a significant period. Milk is a complex biological fluid, characterized by its composition and physical properties that vary across different species, aligning with the specific dietary requirements of their offspring. While water constitutes the primary component of milk, its composition also includes varying amounts of lipids, proteins, and carbohydrates that are synthesized within the mammary gland. Additionally, trace amounts of minerals, fat-soluble substances, water-soluble components, specific blood proteins, and intermediates of mammary synthesis are present in milk, originating from blood plasma **(Alan et *al*., 2001).**

I.2 Milk importance and composition

Milk holds great significance in the human diet on a global scale. However, it is considered one of the most perishable food items due to its ability to provide favorable conditions for the growth of microorganisms. Despite this, milk offers essential nutrients and acts as a source of energy and building materials for growth. It also contains antibodies that protect young mammals from infections. The estimated need for growth can be met by consuming approximately 1000 liters of milk **(Harding, 1999).** The composition of milk varies significantly across different species, influenced by factors such as protein type, protein-fat-sugar proportions, levels of vitamins and minerals, and characteristics like butterfat cell size and curd strength **(Raw Milk Cheese Makers' Association, 2009).**

I.3 Goat's milk

Goat milk is a type of dairy product obtained from the mammary glands of goats. While it shares similarities with cow's milk, there are notable differences in its composition and organoleptic qualities. Goat milk generally contains a slightly higher fat content compared to cow's milk and slightly lower lactose content. It is frequently regarded as more digestible than cow's milk, which can be attributed to the smaller size and distinct structure of the fat globules present in goat milk.

I.3.1 Characteristics of goat milk

1. Flavor and Texture: Goat milk has a distinct flavor that is often described as creamy, slightly sweet, and with a hint of tanginess. The flavor can vary depending on factors such as breed, diet, and processing. In terms of texture, goat milk tends to have a smoother and creamier consistency compared to cow's milk.

2. Digestibility: Goat milk is often considered more digestible than cow's milk. This is partly due to the smaller fat globules and different protein structures present in goat milk, which can make it easier for some individuals to tolerate, especially those with lactose intolerance or dairy sensitivities.

3. Nutritional Profile: Goat milk is a good source of essential nutrients. It contains high-quality proteins, vitamins such as A, B2 (riboflavin), and minerals such as calcium, phosphorus, and potassium. It also provides smaller amounts of other vitamins and minerals like vitamin D, vitamin B12, zinc, and selenium.

4. Lactose Content: Goat milk naturally contains less lactose (milk sugar) compared to cow's milk. This lower lactose content can make goat milk more suitable for individuals with lactose intolerance or those who experience digestive discomfort when consuming cow's milk.

5. Allergenic Potential: Although rare, some individuals may have an allergy to goat milk proteins. People with known allergies to cow's milk should exercise caution when trying goat milk or consult a healthcare professional for advice.

6. Culinary Uses: Goat milk is widely used in various culinary applications. It is commonly consumed as a standalone beverage, used in cooking and baking, and is a popular choice for making cheese, yogurt, ice cream, and other dairy-based products.

It's important to note that individual experiences and preferences may vary when it comes to goat milk. If you have specific dietary concerns or health conditions, it's advisable to consult a healthcare professional or a registered dietitian for personalized advice **(Park, 2019).**

I.4 Ewe's milk

Sheep milk is a consumable liquid produced by the mammary glands of sheep. It stands apart from cow's milk due to its unique composition and characteristics. Notably, sheep milk is renowned for its elevated levels of fats and proteins, which contribute to its creamy texture and distinct flavor profile. Additionally, it is highly regarded for its superior digestibility and lower lactose content compared to cow's milk, making it a preferred choice for individuals with lactose sensitivity. Sheep milk finds extensive application in the creation of diverse dairy products, including cheese, yogurt, and creams **(Salimei, 2012).**

I.4.1 Characteristics of sheep milk

1. Flavor and Texture: Sheep milk has a rich, creamy, and slightly sweet flavor. It is often considered more robust and pronounced compared to cow's milk. In terms of texture, sheep milk has a higher fat content, which contributes to its smooth and velvety consistency.

2. Nutritional Composition: Sheep milk is highly nutritious and known for its high content of fats, proteins, vitamins, and minerals. It contains a higher proportion of solids, including solids-not-fat (SNF), which gives it a creamier texture. Sheep milk is particularly rich in calcium, phosphorus, and vitamins A, E, and D.

3. Digestibility and Lactose Content: Sheep milk is easier to digest compared to cow's milk due to its unique composition. It contains a different protein structure and smaller fat globules, making it more readily absorbed by the digestive system. Additionally, sheep milk has a lower lactose content, which may be more tolerable for individuals with lactose intolerance.

4. Milk Production: Sheep milk production per animal is generally lower than cow's milk production. However, sheep milk has a higher nutrient density, making it valuable for its concentrated nutritional profile.

5. Culinary Uses: Sheep milk is widely used in the production of various dairy products. It is especially favored for making artisanal and specialty cheeses, such as Pecorino Romano, Roquefort, and Feta. Sheep milk is also utilized in the production of yogurt, ice cream, butter, and other dairy-based products.

6. Traditional and Cultural Significance: Sheep milk has been consumed and valued by various cultures throughout history. It has played a significant role in Mediterranean and Middle Eastern cuisines, where sheep milk products have been cherished for their unique flavors and textures.

As with any food, individual sensitivities and preferences may vary. If you have specific dietary concerns or health conditions, it's advisable to consult a healthcare professional or a registered dietitian for personalized advice **(Martin and Dewhurst, 2002).**

I.5 The Common points between goats milk and sheep milk

Goat milk and sheep milk share commonalities as dairy products derived from the mammary glands of goats and sheep, respectively. These kinds of milk distinguish themselves from cow's milk in terms of composition and characteristics. Notably, both goat milk and sheep milk exhibit higher fat content than cow's milk while offering relatively enhanced digestibility and lower lactose content. Furthermore, both types of milk find application in the creation of diverse dairy products, including cheeses and yogurts **(Feng and Zhao, 2019).**

I.6 The difference between goat milk and sheep milk

Goat milk and sheep milk differ in terms of their animal source, composition, and characteristics. Goat milk is obtained from goats' mammary glands, while sheep milk comes from sheep.

Regarding composition, goat milk generally has a higher fat content compared to sheep milk. Goat milk typically contains approximately 3.5-4.5% fat, whereas sheep milk has a higher fat content ranging from 6-8%.

Protein content is another distinguishing factor. Goat milk has a slightly lower protein content, usually around 2.9-3.8%, whereas sheep milk has a higher protein content ranging from 5-6%. Lactose content sets them apart as well. Goat milk has a lower lactose content than cow's milk, making it more easily digestible for individuals with lactose intolerance. Conversely, sheep milk has a lactose content similar to that of cow's milk.

These variations in fat, protein, and lactose content contribute to differences in taste, texture, and potential applications for each type of milk **(Muehlhoff et *al*., 2013).**

II. Chapter Materials and methods

II.1 Objective of the study

The objectives of our study are as follows:

- Investigate and compare the physicochemical properties of both milk types (goat and sheep) in the Tiaret region.
- Analyze the nutritional composition of both milk types (goat and sheep), including nutrients, fats, and proteins.
- Isolation of lactic acid bacteria from goat's and sheep's milk.
- Antibacterial activity of lactic acid bacteria isolated against pathogenic bacteria.

II.2 Duration and location of work

Our experimental study took place at the microbiology laboratory of IbnKhaldoun University, specifically the Faculty of Natural and Life Sciences, in Tiaret. The study was conducted between February 12, 2023, and March 21, 2023.

II.3 Study area

The city of Tiaret is situated in the northwestern mountains of Algeria, specifically in the Tell region. It is surrounded by picturesque mountains and hills, creating a scenic landscape that is complemented by fertile valleys containing lush fields and orchards. The climate of the Tiaret region can be classified as Mediterranean, characterized by hot and dry summers and cool and humid winters. The majority of rainfall occurs between the months of October and May, with an average annual precipitation of approximately 500 mm **(Figure 01).**

The Tiaret region is renowned for its agricultural activities, with a particular emphasis on the breeding of cattle, goats, and sheep. The local farmers have developed expertise in raising goats and sheep, as these animals are well-suited to the climatic and topographical conditions of the region. The region is also notable for its production of traditional dairy products, including cheese and yogurt. The preparation of these dairy products often follows time-honored methods that have been passed down through generations, contributing to the preservation of local culinary traditions.

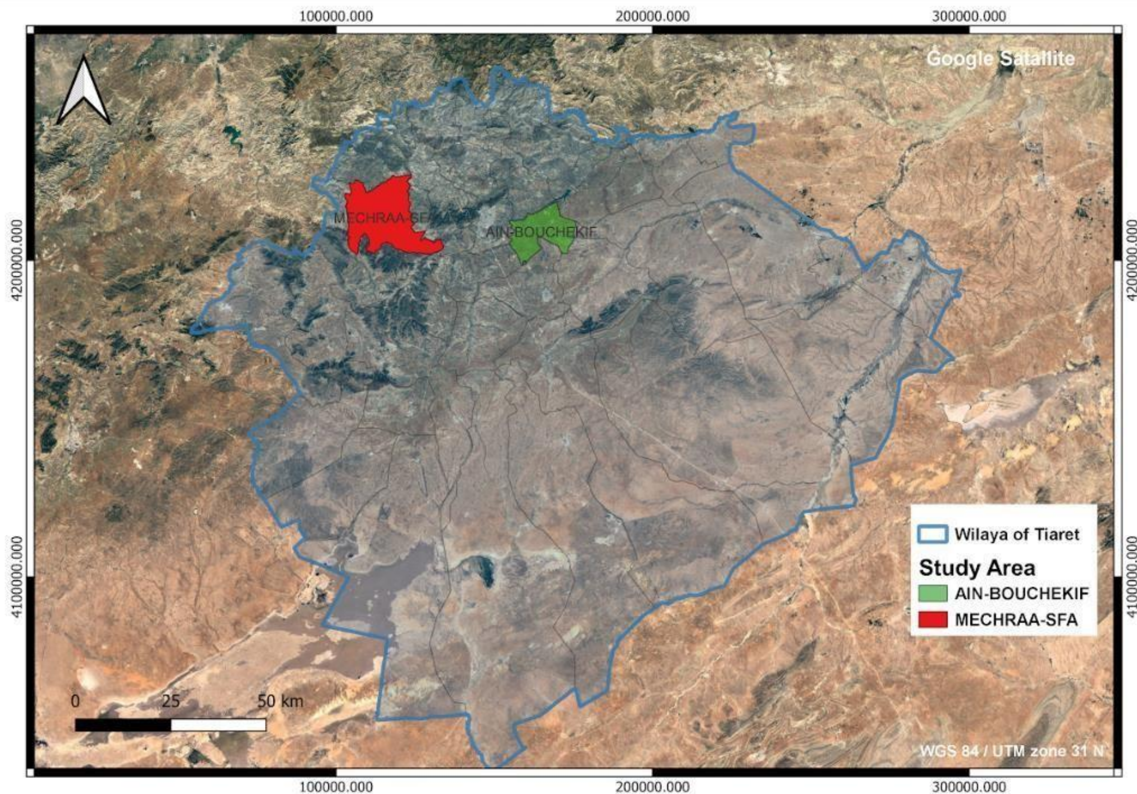


Figure 1: Situation géographique de la province de Tiaret. Source : QGIS 2023

When selecting the study area for the comparison between goat and sheep milk in the Tiaret region, several criteria should be taken into consideration:

1. Availability of goat and sheep farms: It is essential to choose a region where a sufficient number of goat and sheep farms are present. This ensures an ample supply of milk samples for analysis and comparison.

2. Farm diversity: Opting for a region with diverse farms can provide valuable insights. Consider selecting farms with different feeding methods (such as grazing or indoor feeding), various breeds of goats and sheep, or farms of different sizes. This allows for a comprehensive examination of milk variations across different farming practices.

3. Proximity to analysis laboratories: Selecting a region with accessible analysis laboratories capable of conducting the necessary physicochemical and microbiological analyses is crucial. This ensures timely and efficient processing of milk samples and facilitates the collection of accurate data.

4. Consistent environmental conditions: Environmental factors, including climate and geography, can influence milk quality and composition. Choosing a region with relatively

homogeneous environmental conditions helps minimize the impact of these factors on the study's results. This allows for a more accurate comparison between goat and sheep milk, as any variations observed can be attributed primarily to inherent differences between the milk types.

By considering these criteria, the selection of the study area in the Tiaret region can provide a suitable and conducive environment for conducting a robust and meaningful comparison between goat and sheep milk

Based on these criteria, it is feasible to identify multiple study areas within the Tiaret province, considering the unique characteristics of each region. For instance, prioritizing mountainous regions could be advantageous when examining goat and sheep farms that are naturally suited to such environments. These areas provide favorable conditions for grazing and vegetation, which can significantly impact the milk's composition and quality.

II.4 Milk sampling

Milk sampling should be conducted using aseptic techniques to prevent contamination. Before collection, it is essential to ensure that the collection containers are thoroughly cleaned and sterilized. Specifically designed sterile containers intended for microbiological tests and physicochemical analyses should be used for sample collection. To ensure representativeness, the milk samples must be well mixed before collection to minimize any heterogeneity.

To capture potential variations in milk composition, samples can be collected at different times of the day. This allows for an assessment of diurnal fluctuations in milk characteristics. It is crucial to label each collected milk sample with pertinent information, including the date, time, animal type (sheep or goat), and location of the collection. This labeling ensures accurate record-keeping and facilitates traceability throughout the study.

Tableau 1: Sampling locations and characteristics of farms.

Region / Coordinate	Region 01 (Machraa Sfaa)		Region 02 (Ain Bouchakif)	
Animal type	Goat	Ewe	Goat	Ewe
Breed	Saanen	Rembi	British alpine	Rembi
Age	04	02	03	02
Feeding	Barley Wheat Bran Wheat straw Barley	Barley Wheat bran Wheat straw Barley	Barley Wheat bran Wheat straw Barley	Barley Wheat bran Wheat straw Barley
Sampling date	11/02/2023	11/02/2023	11/02/2023	11/02/2023
Number of samples taken	02	02	02	02

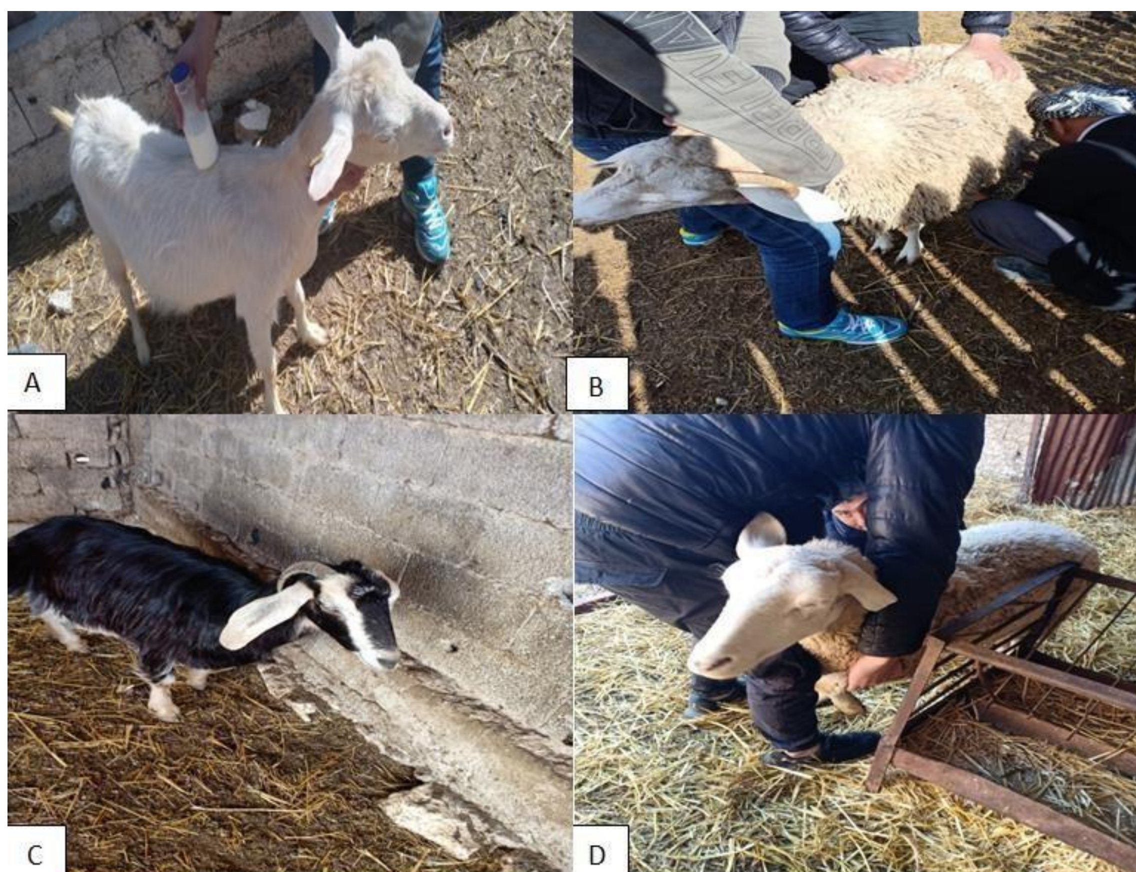


Figure 2: different ewe and goat breeds in the region of MECHRAA-SFA and AIN BOUCHAKIF Region 01 (A-breedSaanenn B- breed remainsi) Region02(C- Breed British alpine breed remains)

Figure 3: Milk Sampling

II.5 Bacterial strains

Pathogenic germs as indicator bacteria were used in the current study as inhibitors of lactic acid bacteria. Three bacterial strains were ATCC reference strains*: Escherichia coli* (ATCC 25922),*Psodomonasaeroginosa*(ATCC 9027)*, Bacillus cereus* (ATCC 14579),and *Staphylococcus aureus*(ATCC 43300),from the Laboratory of Microbiology, Faculty of Natural and Life Sciences, Tiaret University. The purification of pathogenic bacteria was carried out by seeding streaks on the selective media of each pathogenic bacteria. The dishes were incubated at 37°C for 24 hours.

II.6 Probiotic

A product called Bénéflore contains six lactic acid bacteria: Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus bulgaricus, Streptococcus thermophilus, Bifidobacterium longum, Bifidobacterium species, and prebiotics: Maltodextrin, Chicory fructo-oligosaccharides, Pisum sativum fiber (commercialized in France).

II.7 Materials and products

The equipment and products used in our work are illustrated in the following table:

Tableau 2: Equipment and products used in various microbiological

Products	Glasswares	Materials	Culture media	Others
NAOH	Beacher	pH meter	M17	Icebox
PHYNILEPHITALIN	Picnometre	Laboratory Incubator	MRS	Tweezers
H_2O_2	Sterile Pasteur pipette	The precision balance	Mueller-Hinton	Platinum Loop
Distilled water	Petri dish	Autoclave		Blade
Fuchsine	Test tubes	Magnetic stirrer		Micropipette Tips
Crystal violet	Desiccator	Spectrophotometer		
Iodine	Measuring cups	Cell or cuvette		
Ethanol	Graduated pipette	Refrigerator		
Disinfectant	Watch glass	Bunsen burner		
		Optical microscope		

II.8 Protocol followed

In this section, we will discuss the protocol followed in obtaining and analyzing two types of milk.

Figure 4: Protocol follwed

II.9 Physicochemical characterization

II.9.1 Temperature

The temperature at which milk is collected from Saanen, Alpine, and British Alpine goat breeds is generally consistent and aligns with general recommendations. Goat milk is typically collected at the body temperature of the animal, which is approximately 38°C. However, after milking, the milk is rapidly cooled to inhibit bacterial growth during storage. It is customary to cool goat milk to a temperature range of around 4°C to 6°C immediately after milking. This lower temperature is crucial for preserving the milk's quality and freshness **(FAO, 1994).**

II.9.2 pH

pH is a crucial parameter that determines the acidity or alkalinity of a substance, including milk. It plays a significant role in influencing the taste, texture, and shelf life of milk. Generally, the pH of milk falls within the range of 6.4 to 6.8 .

To measure the pH of milk, various methods can be employed, such as using a pH meter or pH test strips. A pH meter is an electronic device that measures the potential difference between a pH-sensitive electrode and a reference electrode, providing a numerical pH value. pH test strips, on the other hand, rely on a color change reaction to indicate the pH level, and the result is compared to a color chart for interpretation **(Noureddine et *al*., 2015).**

When using a pH meter, the following procedure is typically followed:

1. Rinse the pH electrode with distilled water to eliminate any residue or contaminants.

2. Immerse the electrode fully into the milk sample that needs to be tested.

3. Allow the electrode to stabilize in the milk sample for a few seconds until the pH reading on the pH meter becomes stable.

4. Record the pH value displayed on the pH meter, along with any relevant details like the milk's temperature during the measurement.

When employing pH test strips, the procedure is as follows:

1. Pour 1 ml of milk into a container.

2. Immerse the pH test strip into the milk, ensuring it is fully submerged.

3. Wait for 3-5 seconds to allow the color change on the strip to occur.

4. Compare the color change to the provided color chart to determine the approximate pH level.

Although the pH test strip method is an approximate estimation and does not provide an exact numerical result, it can still offer valuable information about the milk's acidity or alkalinity.

It is important to note that accurate pH measurements require proper calibration and adherence to the manufacturer's instructions for the pH meter or pH test strips (Fig.05).

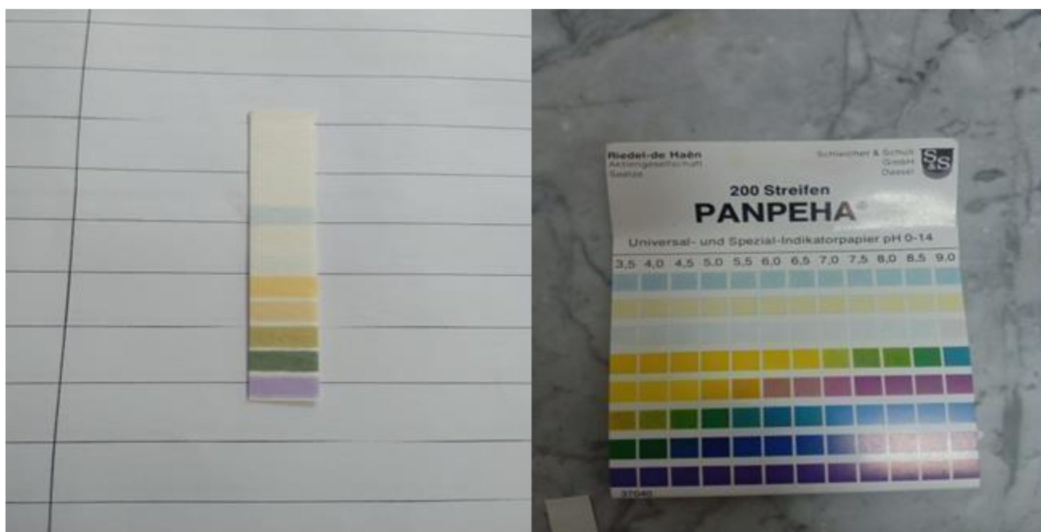


Figure 5: pH test strip

II.9.3 Titrable acidity

As a routine method the total titratable acidity is determined by using the titration method where the milk sample is titrated against standard alkali to the phenolphthalein end point. This corresponds to the pH 8.3 or 8.4. and at this end point the color of the phenolphthalein would change from colourless in the acidic medium to pink in the alkaline medium. The milk is being titrated using the standard sodium hydroxide and the acidity is determined by calculating the acidity using the volume of the standard sodium hydroxide used for the titrating to the phenolphthalein end point. The total titratable acidity of milk is expressed as percent lactic acid. The pH of the milk does not truly represent the titratable acidity because of the non availability of the ionic groups to titration in the normal milk. It is also a matter of interest that the titratable acidity of a milk sample will vary depending upon the buffering capacity of the milk. The natural constituents protect the milk from developing acidity.

Mastitis, even in mild or sub-clinical form, causes the acidity of the milk to be lower. In rare cases mastitis causes a high acidity in the milk **(Wong et *al*., 1988)**

To determine the titratable acidity of milk, the following procedure is typically followed:

1. Prepare a standard sodium hydroxide (NaOH) solution of known concentration. This solution will be used to neutralize the acids in the milk.

2. Weigh a known amount of the milk sample into a beaker.

3. Add a few drops of phenolphthalein indicator to the milk. The indicator will exhibit a pink color in the presence of acid.

4. Slowly add the standard NaOH solution to the milk while stirring continuously until the pink color from the indicator disappears. This indicates that all the acid in the milk has been neutralized.

5. Take note of the volume of the NaOH solution used to neutralize the milk.

6. Repeat the titration process three times and calculate the average volume of NaOH solution used.

By following these steps and calculating the average volume of NaOH solution, the titratable acidity of the milk can be determined, providing valuable information about its acid content.

It is important to ensure accurate measurements by properly calibrating the equipment, using appropriate indicators, and adhering to standard protocols. Additionally, safety precautions should be taken when handling chemicals such as NaOH.

II.9.4 Determination of density

To determine the density of milk using a pycnometer, the following procedure is typically followed:

1. Ensure that the pycnometer is thoroughly cleaned and dried to remove any residual substances that could affect the measurement.

2. Using a graduated pipette, carefully add a known quantity of milk to the pycnometer. Take care to avoid trapping any air bubbles in the milk.

3. Weigh the empty pycnometer using a balance, recording the measurement.

4. Fill the pycnometer with milk and weigh it again, noting the measurement.

5. Calculate the mass of the milk by subtracting the weight of the empty pycnometer from the weight of the pycnometer filled with milk.

6. Determine the volume of the milk in the pycnometer by subtracting the volume of the empty pycnometer from the volume of the pycnometer filled with milk. This can be calculated based on the known volume of the pycnometer.

7. Finally, calculate the density of the milk by dividing the mass of the milk by the volume of the milk in the pycnometer.

By following this operating procedure, the density of the milk can be accurately determined, providing valuable information about its mass per unit volume. It is important to ensure precise measurements by using calibrated equipment and following standard protocols. The cited study by **(Lima et *al*., 2002**) provides additional details and insights into this method of density determination.

II.9.5 Determination of total solids

The method of drying in an oven: it consists of drying a sample of milk in an oven at a constant temperature, and then weighing the resulting dry residue. The difference between the weight of To determine the total solids in milk using the drying in an oven method, follow the operating procedure below:

1. Preheat the oven to a temperature of 102-105°C to ensure a consistent drying environment.

2. Weigh an empty and dry beaker (CAP1) using a balance with a precision of 0.1 g. Record the weight.

3. Add approximately 5 g of the milk sample into the beaker. Take care to accurately measure the sample.

4. Weigh the beaker with the milk sample (CAP2) using the same balance with a precision of 0.1 g. Record the weight.

5. Place the beaker with the milk sample into the preheated oven. Ensure it is securely positioned to prevent any spillage or loss during the drying process.

6. Allow the sample to dry in the oven for 2 to 3 hours or until it is completely dry. The duration may vary depending on the specific conditions and moisture content of the milk sample.

7. Once the drying process is complete, remove the beaker from the oven and place it in a desiccator to cool down. This helps prevent moisture absorption from the surrounding environment.

8. Weigh the beaker with the resulting dry residue (CAP3) using the same balance with a precision of 0.1 g. Record the weight.

9. Calculate the number of milk solids by subtracting the weight of the empty beaker (CAP1) from the weight of the beaker with the dry residue (CAP3). This difference represents the weight of the dry solids in the milk sample.

It is important to note that this method of determining total solids in milk through drying in an oven provides an estimation of the solids content. Standardizing the operating procedure and ensuring accurate measurements are crucial for obtaining reliable results. To determine the total solids in milk using the drying in an oven method, the following operating procedure is typically followed:Preheat the oven to a temperature of 102-105°C.

Figure 6: The precision balance/ Measuring cups

II.9.6 Determination of: fat, protein, lactose, added water, freezing point, salt and conductivity

Lactoscanner is a valuable device in the dairy industry that enables real-time measurement of milk composition using near-infrared spectroscopy (NIRS). This non-destructive technique provides quick and accurate results without the need to destroy the milk sample.

Lactoscanner is capable of measuring several components of milk, including:

1. Fat: The device can determine the fat content in milk, which is an important parameter for determining the richness and creaminess of dairy products.

2. Protein: Lactoscanner can measure the protein content in milk, which is essential for assessing the nutritional value and functionality of milk in various dairy products.

3. Lactose: The device can analyze the lactose content, which is the natural sugar present in milk and plays a role in its taste and texture.

4. Total solids: Lactoscanner can provide an estimation of the total solids content in milk, representing the combined mass of all the components in milk, including fat, protein, lactose, minerals, and other solids.

5. Added water: Lactoscanner can detect the presence of added water in milk, which is crucial for ensuring the authenticity and quality of milk products.

Using the measurements of these components, Lactoscanner can also calculate additional parameters, such as cheese yield and corrected fat content. These parameters are important for dairy manufacturers to optimize production processes and ensure consistent quality in their products.

Lactoscanner is widely used in the dairy industry to maintain quality control and ensure the consistency of dairy products. It provides valuable information for farmers as well, allowing them to monitor milk production from their herd and make informed decisions regarding feed management to improve milk quality.

Overall, Lactoscanner plays a significant role in facilitating efficient milk analysis, supporting quality assurance in the dairy industry, and enabling farmers to optimize milk production.

Figure 7: Lactoscanner

II.10 Isolation of bacteria

To ensure safety and identify potential bacterial strains that may pose a risk to human health, various methods are available for the detection of bacteria in milk. One commonly employed technique in food microbiology is the culture and identification of lactic acid bacteria through microscopy. This method is utilized to assess the composition and quality of dairy products.

The initial step involves the preparation of a milk sample, which is then inoculated into a suitable culture medium. Commonly used culture media for lactic acid bacteria include Man Rogosa Sharpe (MRS) and (M17). Once the bacteria have been cultured, they can be observed under a microscope to analyze their morphology and cellular structure. Lactic acid bacteria are typically classified as Gram-positive, displaying a purple coloration when subjected to the Gram staining method.

Microscopy also allows for the identification of specific characteristics of lactic acid bacteria, such as their shape and cellular arrangement. For instance, certain species can be distinguished based on their spherical (cocci) or elongated (bacilli) shape, as well as their cellular arrangement in chains, pairs, or clusters **(Smith, 2019).**

II.10.1 Dilution of milk

Dilution of milk is a common practice during the identification of bacteria present in milk due to various reasons. Primarily, milk naturally contains a high concentration of bacteria, which can complicate the identification process by making it challenging to pinpoint the specific strains of bacteria being targeted. Dilution serves to decrease the bacterial density, enabling the isolation of individual bacterial colonies for more accurate identification.

Moreover, dilution helps prevent the overgrowth of certain bacterial species that might dominate the culture, potentially masking the presence of other bacterial strains. By diluting the milk, the bacteria are evenly dispersed within the culture, facilitating better observation and identification.

Additionally, dilution plays a crucial role in minimizing errors in identification results by reducing the impact of cross-contamination between samples. It allows for precise counting of bacterial colonies, enhancing the reliability and accuracy of the identification process **(Morgan and Larkin, 2018).**

The standard procedure typically involves the following steps for dilution and identification of bacteria in milk. To begin, a set of sterile test tubes is prepared and labeled in a sequential dilution order. A sterile dilution solution is created using sterile water. A small quantity of the milk sample is transferred into the first test tube, followed by the addition of an appropriate amount of sterile dilution solution. The mixture is thoroughly mixed.

Next, a small portion of the mixture from the first test tube is transferred to the second test tube, and this process is repeated for each subsequent test tube in the dilution series. Each test tube is appropriately labeled to indicate its corresponding dilution level.

The next step involves inoculating the contents of each test tube onto sterile bacterial culture agar plates, such as (MRS) or (M17), using a sterile transfer loop. The plates are then incubated at a temperature ranging from 37°C to 40°C for a period of 48 hours, allowing time for bacterial growth to occur.

Finally, the plates are carefully examined to observe and analyze the bacterial colonies that have developed. These colonies can be further subjected to various identification techniques, such as microscopy, staining methods, or biochemical tests, to determine the specific strains of bacteria present in the milk sample.

II.11 Identification of bacteria

II.11.1 Catalase

Catalase is an enzyme that can be found in various organisms, such as bacteria, plants, and animals. Its main function is to break down hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2) through a decomposition reaction.

Here's the procedure for conducting the catalase test:

- Create a bacterial suspension by taking a small number of bacterial colonies and immersing them in a sterile saline solution.
- Place a drop of this bacterial suspension onto a clean and dry glass slide.
- Add a drop of hydrogen peroxide (H_2O_2) to the bacterial suspension on the slide.
- Observe the formation of gas bubbles (oxygen) within the bacterial suspension.

- If bubbles rapidly form, it indicates the presence of catalase, resulting in a positive outcome.

- If no bubbles or very slow bubble formation occurs, it indicates the absence of catalase, resulting in a negative outcome **(Cappuccino and Sherman, 2016).**

II.11.2 Gram staining

Once we obtain reliable results, we will proceed to the next step, which is the Gram staining.

Here is a basic procedure for Gram staining:

- Prepare a heat-fixed bacterial smear on a clean glass slide.
- Flood the slide with crystal violet for 1 minute.
- Rinse the slide gently with water.
- Flood the slide with iodine for 1 minute.
- Rinse the slide gently with water.
- Decolorize the slide with ethanol for a few seconds until no more color is visible when rinsing the slide with water.
- Rinse the slide gently with water.

- Counterstain the slide with Fuchsine for 1 minute.
- Rinse the slide gently with water.
- Blot dry the slide with bibulous paper.
- Examine the slide under a microscope using an oil immersion lens

After Gram staining, we proceed to streak the bacteria into (MRS) and (M17) agar plates.

II.11.3 Streak

It is an important step in microbiology because it allows isolating a pure bacterial strain from a mixture of bacterial colonies. This is necessary to study a specific bacterial strain and obtain accurate results during tests or experiments. We perform the subculture in both surfaceand depth media, Surface plating allows obtaining a well-defined colony that can be used for further tests, while deep plating allows the isolation of anaerobic bacteria that may be present in the sample

II.11.4 Simmons citrate

The Simmons citrate test is a method used to assess the ability of a bacterium to utilize sodium citrate as the sole carbon source in a culture medium. Here is the general method used for this test:

- Sterilize the inoculation loop by passing it through the flame of a Bunsen burner until it becomes red-hot. Allow it to cool briefly.
- Take an isolated bacterial colony from your pure culture and inoculate it into the test tube containing Simmons citrate culture medium.
- Ensure that the tube is properly labeled for later identification.
- Incubate the tube at the appropriate temperature for the tested bacterial strain, usually at 37°C, for 24 to 48 hours **(Oxoid, 2016).**

II.11.5 The mannitol mobility

The Mannitol Mobility Test is a microbiological test used to determine the ability of bacteria to ferment mannitol, a type of sugar alcohol, and exhibit mobility in a specific culture medium. The test helps in the identification and differentiation of bacterial species based on their metabolic characteristics

Here is the general method used for this test:

- Sterilize the inoculation loop by passing it through the flame of a Bunsen burner until it becomes red-hot. Allow it to cool briefly.
- Retrieve a single isolated bacterial colony from your pure culture using the inoculation loop.
- Inoculate the bacterial colony into the mannitol agar tube by inserting the loop into the medium and streaking it in a zigzag pattern.
- Repeat steps 2 and 3 for each bacterial strain being tested.
- Incubate the mannitol agar tubes at the appropriate temperature for the tested bacterial strain, typically at 37°C, for 24 to 48 hours. **(Murray et *al* ., 2015).**

II.11.6 TSI

TSI (Triple Sugar Iron Agar) test is a microbiological method used for differentiating and identifying bacterial species based on their sugar fermentation, gas production, and sulfur reduction abilities. This test utilizes a specialized medium containing glucose, lactose, sucrose, iron, and a pH indicator. It is divided into three sections: a slant section, a butt section, and a deep section. The TSI test is commonly employed in the identification of enteric bacteria like *Salmonella* and *Escherichia coli.*

Here is a summary of the TSI test procedure:

- Obtain two tubes, one containing an aerobic bacterium and the other containing an anaerobic bacterium. Add specific disks if needed, such as the ONPG disk for the aerobic tube or the oxidase disk for the anaerobic tube, depending on the bacterial strains being tested.
- Sterilize an inoculation loop by heating it in a Bunsen flame until it turns red-hot. Allow it to cool briefly.
- Take a pure bacterial colony of the desired strain and suspend it in sterile distilled water, creating a bacterial suspension.
- Using the inoculation loop, transfer a small amount of the bacterial suspension onto the surface of the TSI medium. Start by inoculating the slant section, then streak the loop downwards into the butt and deep sections to ensure a uniform culture.

- Incubate the TSI tubes at the appropriate temperature, typically 37°C, for a specified period, usually 24 hours. This allows the bacteria to grow and metabolize the sugars in the medium.
- After incubation, carefully observe the TSI tubes for any visible changes and reactions, noting alterations in color, gas production, and growth patterns.
- Record the results and interpret them based on the specific characteristics of the bacterial strains being tested, taking into account color changes, gas production, and the presence or absence of blackening.
- The TSI test utilizes a culture medium containing sugar, iron, and a pH indicator. The observed color changes in the TSI test are as follows:

Yellow: No utilization of glucose and/or lactose in the medium.

Red: Utilization of glucose and/or lactose, resulting in acid production and a decrease in pH.

Black: Utilization of iron to produce hydrogen sulfide (H_2S), leading to blackening of the medium.

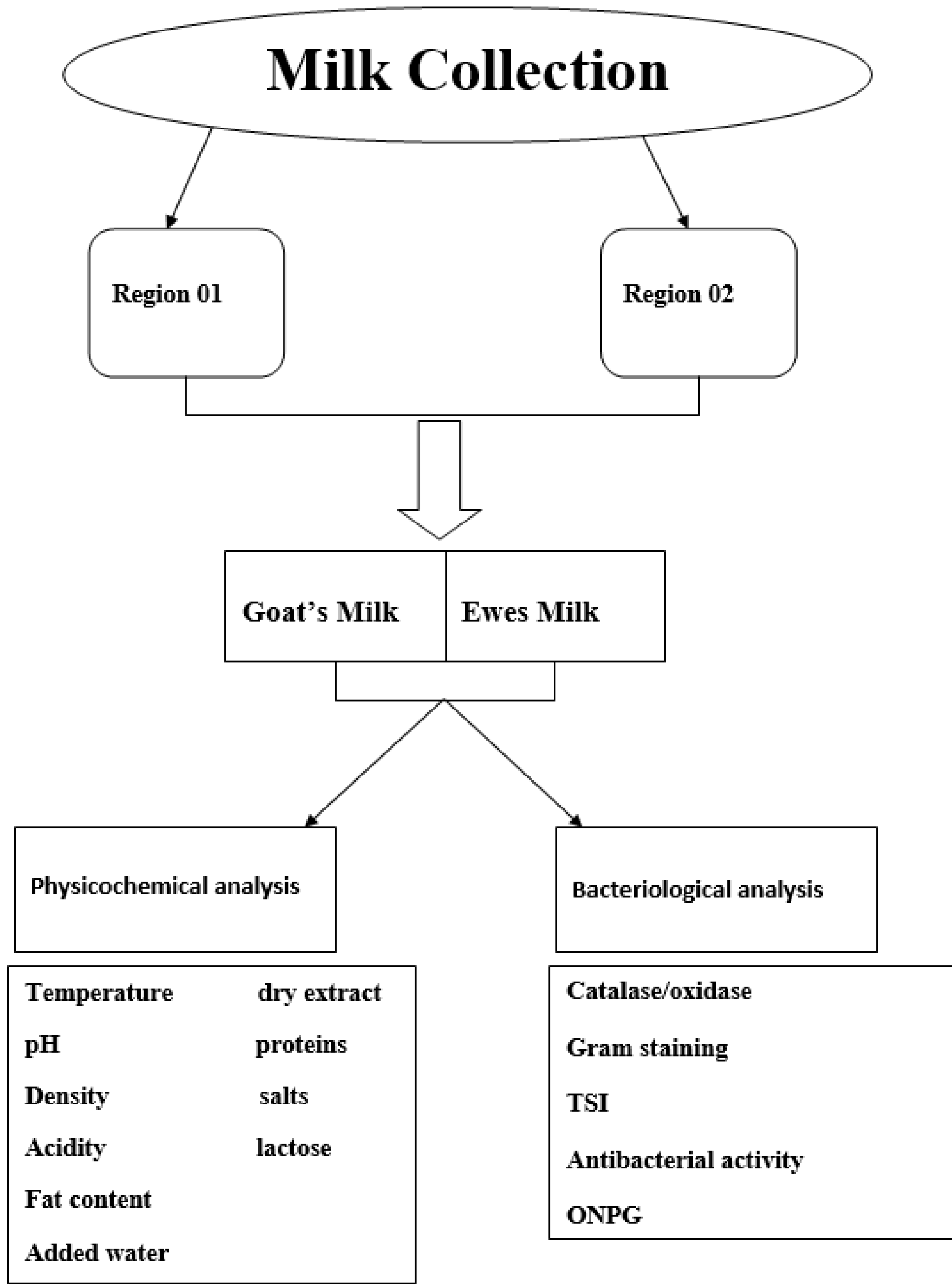
Gas: Production of carbon dioxide (CO_2) and/or hydrogen (H_2) through fermentation of glucose and/or lactose, resulting in gas formation in the medium.

II.11.7 Oxidase test (broth)

The oxidase test is a method used to detect the presence of the oxidase enzyme in bacteria. This enzyme is involved in the electron transport chain of bacteria, and its presence can help differentiate certain bacterial groups.

Here is the general method used for this test:

- Sterilize the inoculation loop by passing it through the flame of a Bunsen burner until it becomes red-hot. Allow it to cool briefly.
- Take a small amount of pure culture from the bacterial sample using the sterile inoculation loop.
- Inoculate the oxidase broth by inserting the inoculation loop containing the bacterial sample into the test tube.
- Ensure good contact between the bacterial sample and the culture medium by gently shaking the test tube.



- Incubate the test tube at the appropriate temperature for the tested bacterial strain, usually at 37°C, for a specified time **(Wayne, 2018).**

II.12 ONPG test

The ONPG test is a method used to detect the activity of the enzyme β-galactosidase in bacteria. ONPG is an artificial substrate that is cleaved by the β-galactosidase enzyme, producing a reactive product of yellow color.

Here is the general method used for this test:

- Prepare a pure bacterial culture to be tested.
- Sterilize an inoculation loop by heating it in a flame until it becomes red-hot. Allow it to

cool briefly.

- Transfer a small amount of the bacterial culture onto a sterile surface.
- Add a disk of ONPG onto the bacterial culture. Mix gently.
- Incubate the mixture at an appropriate temperature, usually 37°C, for a specified time.

II.13 Antibacterial activity

The antibacterial activity of an extract was assessed using the agar diffusion method, as described by (**Celiktas et al., 2007; Sacchetti et al., 2005**)

The antimicrobial properties of lactic acid bacteria were tested against pathogenic bacteria such as Escherichia coli (ATCC 259222), Pseudomonas aeroginosa (ATCC 9027), Bacillus cereus (ATCC 14579), and Staphylococcus aureus (ATCC 43300) Briefly, cells were resuspended in saline (1–2x108 cells/mL for bacteria (0.5 Mc Farland) and spread on the petri dishes of Mueller-Hinton Agar (MH) **(Bouziane et al., 2007).**

To obtain the supernatant, Lactobacillus spp Streptococcus ssp were removed from two Petri dishes and transferred to tubes containing M17 broth medium, also probiotic waq transfered to M17 broth after the tubes weres incubated at 37°C dring 18 h. The mixture was then centrifuged at 3000 rpm for 10 minutes to separate the supernatant and pellet. The empty tubes were filled with the supernatant. Sterile discs with a diameter of 6 mm were impregnated with with 0.5 mL of the supernatant and deposed in to Muller Hinton agar medium, and the probiotic served as a positive control. The antibacterial activity was determined by measuring the diameter of the clear zone (no bacterial growth) around the disc in millimeters, after incubation, measure the diameter of the inhibition zone around each disc using a ruler or caliper. Compare the measured diameters of the inhibition zones with reference values for the target bacterial strain **(Bouziane et al., 2007)**

III. Chapter

Results

and

Discussion

III.1 Physicochemical results

III.2 Tempreture

The results indicate that the temperature measured in both regions after sampling falls within the range of 36 °C to 37°C. This temperature range is consistent with the body temperature of the animal, which is typically around 35°C to 37°C.

The fact that the measured temperatures align with the expected body temperature suggests that the sampling process was conducted accurately and that the milk was collected at a suitable temperature. The similarity in temperatures between the two regions indicates that the milk from both animals was collected under similar conditions.

Maintaining the appropriate temperature during milk collection is crucial for preserving the quality and preventing bacterial growth. Milk obtained at temperatures close to the animal's body temperature is considered to be in the ideal range, as it reduces the risk of microbial contamination and helps maintain the freshness of the milk.

By ensuring that the milk is collected at an appropriate temperature, it is more likely to be free from temperature-related issues that can affect its quality and safety. These results provide reassurance that the milk samples were handled properly and that subsequent analyses can be conducted with confidence **(FAO, 1994).**

III.3 pH

The Following table compares the pH of goat and sheep milk in two regions of Tiaret, Algeria, with the recommended pH standard for milk.

Tableau 3: *pH values of milk from two regions*

Milk / Region	Goat Milk	Sheep Milk
Machraa Sfa	6.42	6.21
Ain Bouchakif	6.45	6.30
pH norms	6,45 - 6,60	6,5 - 6,85

The results of the study indicate that the pH of goat milk is slightly higher than that of sheep milk in both regions. The pH values observed were 6.52 for goat milk and 6.68 for

sheep milk, on average. This difference in pH between the two types of milk was more pronounced in the second region.

These findings align with the comparative results reported by **(FAO, 2019)** The reference table provided in the publication confirms the pH differences between goat and sheep milk.

Comparing the pH values obtained in the study with the established standards, it is evident that the pH levels of both goat and sheep milk from both regions fall within the acceptable ranges. This compliance with the pH standards suggests that the milk samples analyzed meet the expected quality criteria.

Monitoring the pH of milk is important as it can influence various factors such as taste, microbial growth, and product stability. By adhering to the acceptable pH ranges, the milk samples can be deemed suitable for consumption and further processing.

These results support the consistency of the study findings with established references and demonstrate that the milk samples analyzed in the study comply with the pH standards for goat and sheep milk.

III.4 Acidity

The acidity value is calculated using the following formula.

$$= \frac{[C_{NAOH}] \times V_{NAOH\ utl} / V_{MILK}}{0,1}$$

The obtained values for titratable acidity in both goat milk and sheep milk from the two regions are compared to the accepted norms provided by **(FAO, 2019).**

According to the reference table, the accepted norms for titratable acidity are 14-18 °Dornic for goat milk and 22-25 °Dornic for sheep milk.

Based on the results of the study, it is determined that both goat milk and sheep milk samples from the two regions fall within the acceptable ranges for titratable acidity. This indicates that the milk samples conform to the established norms for titratable acidity, as outlined in the reference publication.

Compliance with the accepted norms for titratable acidity is important as it influences the taste, microbial stability, and overall quality of the milk. The fact that the milk samples meet the titratable acidity standards suggests that they can be considered suitable for consumption and further processing.

Overall, the conformity of the milk samples with the acceptable ranges for titratable acidity reinforces the quality and reliability of the findings in the study, demonstrating compliance with the established norms for goat milk and sheep milk.

Tableau 4*: Results of the acidity of goat and sheep milk.*

Regions / Type of milk	R1	R2	Norms of titrableacidity (°Dornic)
Goat milk	20	21	14-18
Ewe milk	24	17	22-25

III.5 Density

The density values of the analyzed samples of goat and sheep milk are given in the table below:

Tableau 5: Values of density found for goat and sheep milk

Region	1	2	Norms
Milk of goat's (g)	1.036	1.031	1 ,026 to1,042
Milk of ewe's (g)	1.033	1.039	1,034 to1,039

Upon analysis, it is observed that all the milk samples, both from goats and sheep in both regions, conform to the norms mentioned in the reference publication **(FAO, 2019).**

Specifically, focusing on density measurements, it is found that the obtained results for goat and sheep milk in both regions fall within the recommended normal range of 1.026 to 1.042 g/mL. This indicates that the density of the milk in both regions complies with the established quality standards and can be considered acceptable.

It is important to note that variations in milk density may signify differences in milk composition, which can have implications on its properties and overall quality. Therefore,

regular density measurements are necessary to monitor and ensure the ongoing quality of the milk produced in these regions.

By conforming to the recommended density range, the milk samples demonstrate consistency and adherence to the established standards. This implies that the milk can be deemed suitable for consumption and further processing, meeting the expected quality criteria.

Overall, the compliance of the milk samples with the density standards contributes to the assurance of their quality and reinforces the reliability of the findings in the study.

III.6 Chemical result

III.7 Total solids content

the dry matter percentages that were found are represented in the following table

Tableau 6: Percentages of dry matter in goat and sheep milk

Region	Goat milk - Dry matter (%)	Sheep milk - Dry matter (%)
1	18	23,24
2	24,14	36.58
Norms	11% to 18%	16% to 20%

Norms references ; **(Hartmut , 2001) (Konca , 2017)**

The dry matter content of goat milk and sheep milk from two different regions is compared to the general norms, and there are variations observed in the results.

For goat milk in region 1, the dry matter content of 18% falls within the general norms ranging from 11% to 18%. However, in region 2, the dry matter content is higher at 24.14%, exceeding the upper limit of the general norms. It should be noted that it is not uncommon for the dry matter content of goat milk to exceed the upper limit, as it can vary depending on factors such as animal breed and feed.

In the case of sheep milk, the dry matter content in region 1 is 23.24%, which is higher than the general norms ranging from 16% to 20%. Region 2, however, shows a significantly higher dry matter content of 36.58%, exceeding the general norms by a considerable margin.

Several reasons can contribute to the dry matter content being higher than the norms. Firstly, natural variation among milk productions is possible, where certain milk may naturally have higher dry matter content due to factors such as animal feed, breed, and lactation period.

Measurement errors could also be a factor, as inaccurate laboratory measurements or poor measurement techniques can lead to incorrect results. It is mentioned that the experiment was repeated three times, but it is still possible for errors to occur.

Contamination is another possibility, where the presence of foreign materials like dissolved solids from equipment disinfection can influence the dry matter content of the milk.

Considering the variations observed, it would be beneficial to conduct further investigations to determine the specific reasons behind the higher dry matter content in the milk samples from region 2. This could involve additional analysis, quality control measures, and a thorough evaluation of the factors that may contribute to the variations in dry matter content

III.8 Lactoscan results

This table shows the results of the analysis of different biochemical parameters of goat and ewe milk in two different regions.

Looking at the results, it can be seen that the fat content is lower than the standard in region 01 for goat milk, but it is normal for ewe milk. For region 02, the fat content is normal for both types of milk.

The protein content is slightly below the standard for goat milk in both regions, while for ewe milk, it is normal.

The lactose content is within the standard for both types of milk and in both regions.

The presence of added water is zero for all analyses.

The freezing point is lower than the standard for all analyses, but this is due to the fact that the animals were raised in mountainous areas where it is cold.

The salt content is slightly above the standard for ewe milk in region 02, while for other analyses, it is within the standard.

The conductivity is also within the standard for all analyses, although the value is slightly higher for goat milk in Region 01.

Overall, it can be said that the results are generally in conformity with the standards, although **(FAO.2019)** there are some variations depending on the regions and types of milk

Tableau 7: Biochemical caractiristic results

Regions / Parameter / Animals	Regions 01		Regions 02		General Standards
	Goats	Ewes	Goats	Ewes	
Fats g/l	04.71	06.91	05.82	08.83	Minimum 3.5 g/L
Protein g/l	03.66	03.50	03.60	04	Minimum 2.9 g/L
Lactose g/l	05.52	05.97	05.45	06.09	4.5-5.2 g/L
Added water	00	00	00	00	Not allowed
Freezing point °C	-00.66	-00.69	-00.65	-00.77	-0.53 to -0.56°C
Salt g/l	00.82	00.85	00.81	00.91	Maximum 1.2 to 1.5 g/L
Conductivity mS/cm	05.82	05.12	05.21	04.06	Less than 5.5 mS/cm

III.9 Results of lactic acid bacteria isolation

Dilution is a technique employed to reduce the number of microorganisms present in milk and achieve an optimal concentration for microbiological analysis. In this study, dilutions of 1/2 and 1/4 were utilized to decrease the microbial load in the milk samples. Subsequently, 3 to 5 drops of the diluted milk were inoculated onto (MRS) and (M17) culture media. To create anaerobic conditions, the plates were placed in a desiccator with a candle, eliminating oxygen. The plates were then incubated for 48 hours to allow bacterial growth.

Following the incubation period, bacterial colonies were observed on the (MRS) and (M17) plates, indicating the presence of microorganisms in the milk samples under analysis. The quantity and characteristics of these bacterial colonies will provide insights into the level of contamination and the types of microorganisms present in the milk. However, further analysis is required to identify the specific bacteria and determine their potential impact on the quality of the milk.

By employing dilution and microbiological techniques, this study aims to assess the microbial status of the milk samples and gain a deeper understanding of their microbiota. Such information is crucial for evaluating milk quality, ensuring consumer safety, and implementing appropriate measures to control and prevent microbial contamination.

Figure 8: Biochemical caractiristic results

III.10 Catalase

The catalase test is a widely used procedure in microbiology to differentiate between bacteria that possess catalase enzyme activity (catalase-positive) and those that do not (catalase-negative). In this experiment, a few drops of catalase solution were added to 48 plates containing bacterial samples.

Upon observation, the formation of bubbles was noticed in two of the plates. This indicates that the catalase enzyme present in the solution was active and capable of breaking down hydrogen peroxide into water and oxygen. The bubbling reaction serves as a visual confirmation of catalase activity.

Based on the positive catalase reaction, the next step in the analysis involved performing the Gram staining technique. Gram staining is a differential staining method used to categorize bacteria into Gram-positive and Gram-negative based on differences in their cell wall structure. This additional step helps further characterize and identify the bacteria present in the samples.

By combining the catalase test with the Gram staining technique, microbiologists can gain valuable insights into the nature and classification of the bacteria under investigation. This information is crucial for understanding their properties, behavior, and potential implications in various microbiological contexts.

III.11 Gram staining

III.11.1. Examination microscopic

When examining the Gram-stained bacteria under the microscope using cedar oil immersion at a magnification of 100x, the observed bacteria appeared purple in color. The shape of the bacteria varied depending on the genus. For *staphylococci*, the bacteria were coccus-shaped (spherical) and were observed to be arranged in clusters. *Staphylococci* are known for their characteristic grape-like cluster arrangement.

On the other hand, LactoBacillus bacteria were observed to be arranged in chains. *Bacillus* bacteria are rod-shaped (bacillus) and often form chains due to their division pattern.

These observations provide valuable information about the morphology and arrangement of the bacteria. Gram staining, along with microscopic examination, aids in the identification and classification of bacteria, based on their staining characteristics, and cell morphology.

After performing the Gram staining procedure and examining the stained bacterial cells under a microscope, the next step involves interpreting the results. Gram staining is a fundamental technique in microbiology that helps classify bacteria into two main groups: Gram-positive and Gram-negative, based on their cell wall composition.

In this case, the observation that both bacteria stained violet indicates that they are Gram-positive. Gram-positive bacteria retain the crystal violet stain and appear purple or violet under the microscope. This staining pattern suggests that the bacteria have a thick peptidoglycan layer in their cell walls, which retain the violet dye during the staining process. The identification of the bacteria as Gram-positive provides important information about their cell wall structure. Gram staining results, in conjunction with other characteristics such as cell shape and arrangement, contribute to the initial identification and classification of bacteria. Further tests and analysis can be conducted to narrow down the specific genus and species of the Gram-positive bacteria under investigation.

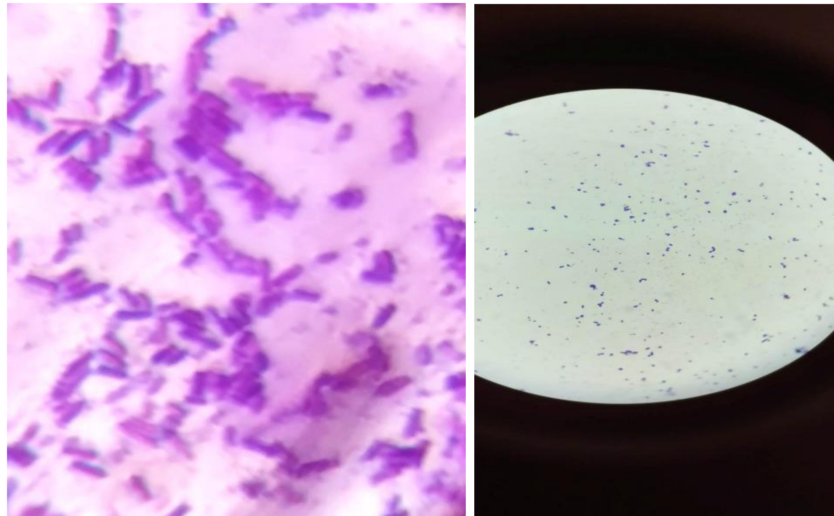


Figure 09: *Lactobacillus/ Streptococcus*

Tableau 8: Forms and Gram staining of isolats

Bacteria	Gram	Form
Streptococcus	+	Pairs of cocci (spherical cells)
Lactobacillus	+	Bacils

The table presents different bacterial species, their corresponding Gram staining results, and cellular forms. Gram staining is a widely used laboratory technique that aids in the classification of bacteria into two major groups: Gram-positive and Gram-negative. This classification is based on variations in their cell wall structures.

According to the table, the bacteria species *"Streptococcus"* is Gram-positive (+) and exhibits a cellular form of pairs of cocci, which are spherical cells. Gram-positive bacteria possess a thick layer of peptidoglycan in their cell walls, enabling them to retain the violet dye during Gram staining and resulting in a positive Gram stain.

Likewise, the bacteria species "*Bacilli*" is also Gram-positive (+) and demonstrates a cellular form of Bacilli, which are rod-shaped cells. *Bacilli*, similar to *Streptococcus,* possess a peptidoglycan cell wall that retains the violet dye during Gram staining, leading to a positive Gram stain.

By performing Gram staining and observing the cellular forms along with the corresponding Gram staining results, it becomes possible to gain insights into the cell wall

composition and classification of bacteria. These findings serve as a crucial first step in

bacterial identification and further characterization **(Murray et *al* ., 2019).**

III.12 Results of biochemical identification of isolates

The Simmons citrate test is a useful tool for identifying and differentiating bacteria based on their ability to utilize citrate. By observing the lack of change in color of the medium from green to blue, we determined that the tested bacteria cannot utilize citrate as a carbon source. This negative reaction rules out the potential identification of the bacteria as those capable of citrate utilization.

In the Mannitol Mobility Test, a negative result is indicated by no color change in the medium from red to yellow. This lack of change occurs because there is no production of acidic metabolites during mannitol fermentation. Bacteria that cannot ferment mannitol do not produce acid, thus maintaining the pH of the medium and resulting in no color change.

TSI (Triple Sugar Iron) test involves incubating the TSI agar plates to allow bacterial growth and sugar metabolism. The observed characteristics include a color change from red to yellow, indicating acid production from sugar fermentation. Additionally, blackening of the medium occurs when bacteria produce hydrogen sulfide (H_2S) as a metabolic byproduct, forming ferrous sulfide. These color changes and blackening patterns help in identifying and differentiating bacterial species.

The oxidase test is performed by reacting the bacterial cells with a reagent, and a negative result is indicated by no color change or the absence of a dark bue-purple color. This indicates the absence of the enzyme cytochrome C oxidase, which is involved in aerobic respiration. Bacteria lacking this enzyme cannot respire using oxygen as a terminal electron acceptor.

Regarding the ONPG (O-Nitrophenyl-β-D-galactopyranoside) test, no observations were made, suggesting a negative result in *streptococcus* . and a positive result was observed in Lactobacillus, this test is typically used to determine the ability of bacteria to hydrolyze lactose (table 9). This test is commonly utilized to assess the presence of the enzyme β-galactosidase, which is responsible for lactose hydrolysis..

Overall, these tests provide valuable information for the identification and characterization of bacteria based on their biochemical and metabolic properties **(Willey et *al*., 2019).**

Tableau 9: Biochemical identification of isolates results

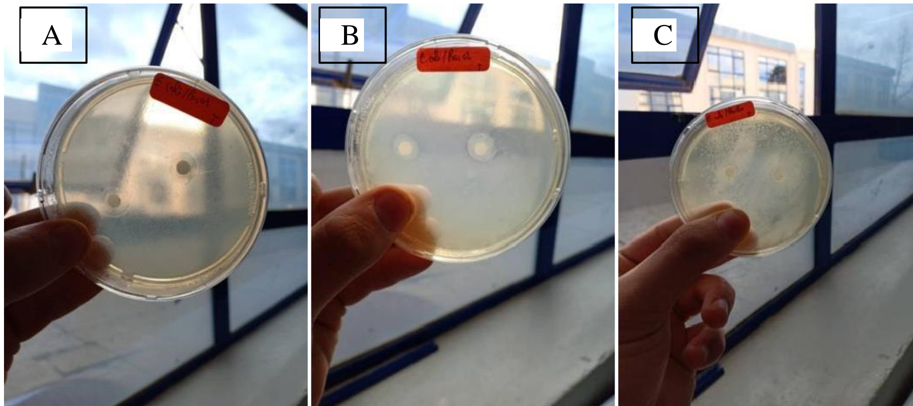
Parameter	*Lactobacilli*	*Streptococcus*
Simmons citrate	-	-
The Mannitol Mobility	-	-
TSI	+	+
Oxidase	-	-
ONPG	+	-
Catalase	-	-

III.13 Antibacterial activity results

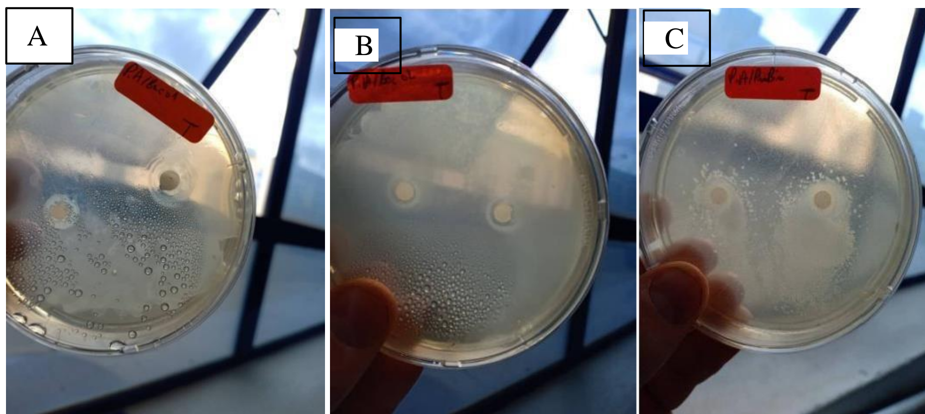
Tableau 10: Antibacterial activity of lactic acid bacteria against pathogenic strain

LAB / Pathogenic	*Streptococcus* sp	*Lactobacilli* sp	(-) *Probiotic*
Escherichia coli	Sensitive (+) 12mm	Sensitive (+) 15mm	Resistant (-) 2mm
Pseudomonas aeruginosa	Sensitive (+) 13mm	Sensitive (+) 11mm	Resistant (-) 6mm
Staphylococcus aureus	Resistant (-) 2mm	Resistant (-) 0mm	Contamination
Bacillus cereus	Resistant (-) 1mm	Resistant (-) 0mm	Resistant (-) 2mm

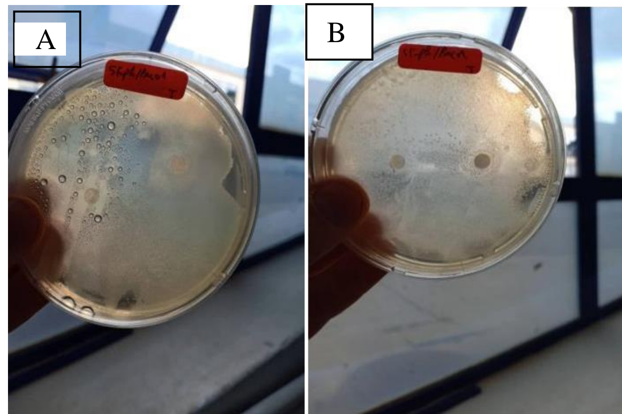
Escherichia coli



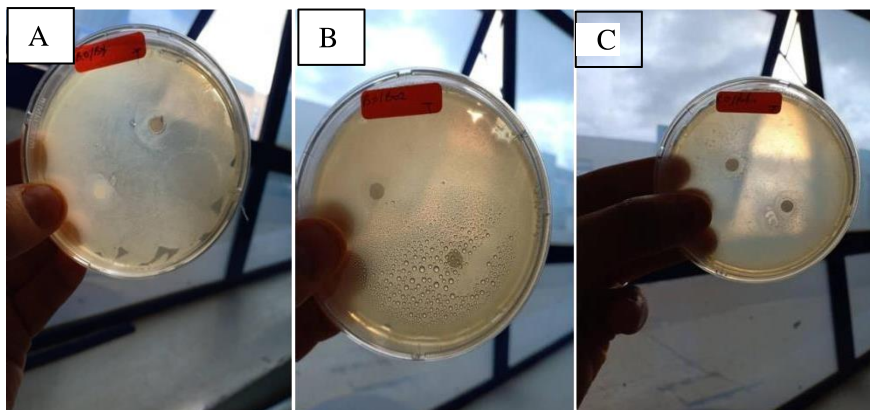
Pseudomonas aeruginosa



Staphylococcus aureus



Bacillus cereus



A: *Streptococcus*

B: *Lactobacilli*

C: Probiotique

Zone Diameter

- Sensitive: The zone diameters of sensitive lactic acid bacteria can vary typically from 10 mm to 30 mm or more, depending on the concentration and effectiveness of the tested antibacterial agent.
- Resistant: Resistant lactic acid bacteria may show either no zone of inhibition (0 mm) or a very limited zone of inhibition, typically less than 10 mm.

The antibacterial activity of lactic acid bacteria (LAB) is well-documented and widely studied. LAB are a group of beneficial bacteria that naturally occur in various environments, including the gastrointestinal tract, plants, and fermented foods. They play a crucial role in food fermentation processes and have been traditionally used to preserve and enhance the safety and quality of food products **(Corsettiet Settanni , 2007).**

One of the main mechanisms by which LAB exhibit antibacterial activity is through the production of organic acids, particularly lactic acid. LAB ferment carbohydrates present in their environment and convert them into lactic acid, lowering the pH of the surrounding medium. The acidic environment created by lactic acid inhibits the growth of many pathogenic and spoilage bacteria, as these organisms are sensitive to low pH conditions. This acidification mechanism is a key factor in the preservation of fermented foods **(Holzapfel et *al* ., 2001).**

In addition to organic acids, LAB also produce various antimicrobial compounds, including bacteriocins, hydrogen peroxide, diacetyl, and reuterin. Bacteriocins are proteinaceous antimicrobial peptides that are produced by certain strains of LAB. These peptides have the ability to inhibit the growth of closely related or even unrelated bacterial strains. Nisin, produced by *Lactococcus* lactis, is one of the most well-known bacteriocins and is widely used as a natural preservative in the food industry (**Parvez et *al*., 2006**).

LAB can also compete with pathogenic bacteria for nutrients and adhesion sites in the gastrointestinal tract, preventing the colonization and growth of harmful microorganisms. They can produce enzymes such as proteases and lipases that degrade proteins and lipids, making the environment less favorable for pathogens **(Salminen et *al* ., 2004).**

Moreover, LAB have been shown to stimulate the immune system and enhance host defense

mechanisms. They can modulate the production of cytokines and other immune factors, leading to improved immune response against pathogens.

The antibacterial activity of LAB has significant implications in various fields, including food preservation, probiotic development, and human health. LAB-based probiotics have been extensively studied for their potential health benefits, including their ability to prevent and treat gastrointestinal infections, improve gut health, and boost the immune system **(Servin and Coconnier , 2003).**

However, it is important to note that the antibacterial activity of LAB is strain-specific, and not all LAB exhibit the same level of antimicrobial activity.

Different LAB strains may produce different types and amounts of antimicrobial compounds, and their efficacy can vary depending on the target pathogen. Therefore, careful selection of LAB strains with proven antibacterial activity is crucial for their practical applications **(Tharmaraj and Shah , 2003).**

Overall, the antibacterial activity of lactic acid bacteria is a valuable characteristic that contributes to their role in food preservation, probiotic development, and promoting human health. Further research is needed to explore the specific mechanisms of antibacterial action and to identify new strains and compounds with enhanced antimicrobial properties

(Vinderola et *al* ., 2019).

IV. Conclusion

Conclusion

Throughout our collaborative study on goat's milk and ewe's milk, we have examined and compared various aspects of these two types of milk. We focused on factors such as composition, pH levels, and titratable acidity.

Based on the collected data and analysis, we found that there are notable differences between goat's milk and ewe's milk. Goat's milk generally exhibits a slightly higher pH and lower titratable acidity compared to ewe's milk. These variations can contribute to differences in taste, texture, and overall quality of dairy products made from these milks.

It is important to note that individual variations can exist within each type of milk, as factors such as breed, diet, and farming practices can influence milk composition. Therefore, it is crucial to consider these variables when studying and comparing goat's milk and ewe's milk.

Our collaborative study has provided valuable insights into the characteristics and properties of goat's milk and ewe's milk. However, further research and analysis are needed to delve deeper into specific aspects such as nutritional content, microbiological aspects, and sensory attributes.

Overall, our collaborative work has enhanced our understanding of goat's milk and ewe's milk, highlighting their unique qualities and aiding in making informed decisions regarding their utilization in dairy products.

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VI. Annexes

Annexes

Preparation of MRS medium:

Table 11 : MRS medium (Man, Rogasa and Sharpe) (pH 6.5)

Peptone	10g
Yeast extract	5g
meat extract	10g
Glucose	20g
Tween 80	1ml
Dipotassium phosphate	2g
sodium acetate	5g
Ammonium citrate	2g
Magnesium sulfate	0.2g
Agar	15g
Distilled water qsp	1000ml

The medium is sterilized at 121° C. for 15 min.

Preparation of M17 medium

Table12: Medium M17 (pH 7.2)

Tryptone	2.5g
Meat peptic peptone	2.5g
Soy papain petone	5g
Yeast extract	2.5g
meat extract	5g
Lactose	5g
Sodium glycerophosphate	19g
Magnesium sulfate	0.25g
Ascorbic acid	0.5g
Agar-agar	15g

The medium is sterilized at 121° C. for 15 min.

pH 7 Autoclaving 120°C/20 minutes

Annexes II

Figure 10: beaker for the titrable acidity and pycnometer for the density