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:Presented by

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Topic

Studies on some functional dairy products

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Attestation de collaboration

Nous, soussignés, attestons par la présente la collaboration entre les étudiants des deux masters suivants pour le projet d'étude intitulé : **"Studies on Some Functional Dairy Products"**, encadré par Professeur TABAK Souhila.

Objet de la collaboration

Cette collaboration vise à combiner les compétences et les connaissances des étudiants des deux masters pour mener à bien une recherche approfondie sur les produits laitiers fonctionnels. Les étudiants travailleront ensemble pour explorer les aspects microbiologiques, toxicologiques et de sécurité alimentaire de ces produits, afin d'améliorer la qualité des produits laitiers disponibles sur le marché.

Étudiants impliqués

- Kouadria Mohamed (Master Microbiologie Appliquée)
- Rezzag Bilal (Master Toxicologie et Sécurité Alimentaire)
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Fait le : .

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Our feelings of gratitude are extended to Mrs. TABAK for her guidance and patience.

We would like to extend our gratitude to the honorable Mrs. BOUBAKEUR, jury president, and the rest of the respected jury members.

And last but not least our dear friends: YOUNES, ADIL, HABIB and ABD RAHMAN in no particular order.

List of abbreviations

Subsp: subspecies

LAB: Lactic acid bacteria

pH: Hydrogen potential

°D: Dornic Degree

MRS: De Man, Rogossa and Sharpe

AOAC: Association of Official Analytic Chemists

E. coli: Escherichia coli

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Summary

INTRODUCTION

INTRODUCTION

Milk is a food rich in nutrients. Dairy industries utilize it in dairy manufacturing through technological processes like lactic fermentation. One aspect involves ensuring food security through acidification and the production of bacteriocins to inhibit the growth of pathogenic bacteria (**Hadjimbei *et al.*, 2022**). Another part entails producing aromatic molecules to improve the dairy product's organoleptic quality. Yogurt, in particular, is considered a functional food due to its probiotic and nutritional properties conferred by the lactic acid bacteria present (**Gómez-Gallego *et al.*, 2018**).

However, it would be wise to determine exactly what makes yogurt a functional food and what differences there may be between homemade yogurt with minimal processing compared to store-bought brands that undergoes more extensive processing and contain additives.

Our objective is to conduct a study focusing on the physicochemical and microbiological characteristics of specific types of yogurts that are most commonly consumed by the population. According to our survey

Our thesis consists of the following three sections:

- First, a literature review section that addresses the fundamentals and general concepts of yogurt followed by a review of our survey on yogurt

- Second, a survey discussion
- Third, an experimental section that focuses on the physicochemical and microbiological characteristics of yogurt.

Literature study

I-1-Définition of a functional food

Functional foods belong to a category of food products designed to provide health benefits that extend beyond their basic nutritional value. They are formulated to contain substances or live microorganisms at safe and effective concentrations, aiming to achieve specific health-enhancing effects (**Temple, 2022**).

I-2-Definition of Yogurt

A fermented milk product is produced through the bacterial fermentation of heat-treated milk, facilitated by the symbiotic interaction of Streptococcus thermophilus and *Lactobacillus delbrueckii* subsp. *bulgaricus* (**CXS 243-2003**).

I-3- Key attributes of yogurt's lactic ferments

I-3-1- *Streptococcus salivarius subsp. thermophilus*

This microorganism is exclusively isolated from milk and milk products. It is characterized as a gram-positive coccus, non-mobile, thermoresistant, sensitive to methylene blue and antibiotics. It is homofermentative, anaerobic, and may occur in the form of chains of variable length or in pairs. The optimal temperature for its growth typically ranges between 40 and 50 °C. Its primary function lies in the fermentation of lactose into lactic acid (**Lagunas *et al.,* 2022**).

Moreover, it plays a crucial role in determining the texture of fermented milks by increasing milk viscosity through the production of polysaccharides. These compounds consist of galactose and glucose, with minor amounts of rhamnose, arabinose, and mannose. In addition to its acidifying properties, these polysaccharides contribute significantly to the texture enhancement of fermented milk products (**Ramize Hoxha *et al.,* 2023**).

I-3-2-*Lactobacillus delbrueckii* subsp *bulgaricus*

This bacterium is a gram-positive bacillus, non-motile, and asporogenic, typically isolated in the form of thermophilic chain rods. It exhibits microaerophilic characteristics and is highly dependent on calcium and magnesium for its growth. The optimal temperature for its growth is approximately 44°C, and it possesses a homofermentative metabolism.

Significantly, this bacterium plays a crucial role in shaping the organoleptic and hygienic qualities of yogurt, underscoring the importance of understanding its functions in yogurt production (**Ramize Hoxha *et al.,* 2023**).

I-4-Importance and functions of yogurt bacteria

I-4-1-Lactic acid production

Lactic acid bacteria (LAB) play a pivotal role in yogurt production by facilitating the generation of acidity, a fundamental aspect for yogurt formation. As LAB ferments the lactose present in milk, it produces lactic acid, leading to a gradual accumulation of acidity and subsequent reduction in pH. This increase in acidity triggers the destabilization and coagulation of milk proteins, ultimately culminating in the development of a dense, gel-like texture characteristic of yogurt (**Ramize Hoxha *et al.,* 2018**).

I-4-2- Texturing activities

The textural characteristics of yogurt are influenced by several factors, one of which is the utilization of exopolysaccharides produced by starter cultures during fermentation. These exopolysaccharides play a crucial role in enhancing the texture of yogurt by influencing parameters such as firmness, cohesiveness, and viscosity.

The presence of these compounds significantly contributes to the overall sensory experience and mouthfeel of yogurt, thereby elevating its quality and enhancing its appeal to consumers (**Xue Han *et al.,* 2016**).

I-4-3- Proteolytic activity

Yogurt's proteolytic activity stems from the proteolytic systems of lactic acid bacteria (LAB) that remain active during fermentation. These LABs produce enzymes responsible for breaking down proteins, including casein and whey proteins, into smaller peptides and amino acids. This proteolysis process significantly influences the texture, flavor, and nutritional quality of yogurt (**Elahe *et al.,* 2016**).

Moreover, LABs play a vital role in hydrolyzing major dairy proteins, thereby affecting the digestibility and bioavailability of proteins in yogurt. These enzymatic activities contribute to the overall sensory experience and nutritional profile of the yogurt product (**Koichiro Sumi *et al.,* 2023**).

I-4-4- Aromatic activity

The aromatic activity observed in yogurt is closely linked to the presence of volatile aromatic flavor compounds, which play a pivotal role in shaping its distinctive aroma and taste profile. These compounds are essential for enhancing the organoleptic qualities of yogurt, significantly influencing its overall sensory experience (**Ayivi *et al.*, 2023**)

The volatile aromatic compounds present in yogurt are paramount for its flavor, contributing to a pleasant and appealing aroma that enhances consumer enjoyment. However, it's worth noting that the presence of these compounds can be influenced or altered by the addition of aromatics, potentially modifying the overall flavor profile of the yogurt product (**Albert Krastanov *et al.*, 2023**).

II-1-Objectives

To assess the safety of the yogurt brands included in our survey and to ascertain their compliance with microbiological and physicochemical standards as outlined in Annex II of the interministerial decree of June 25, 2020, we undertook the following actions:

1. Physicochemical Parameter Control:

- Conducted a comprehensive analysis of physicochemical parameters, focusing on pH and acidity levels, to ensure adherence to regulatory requirements.

2. Yogurt Production:

- Produced our own yogurt samples to serve as benchmarks for comparison against commercial brands, allowing for a more thorough evaluation of quality and safety.

3. Microbiological Quality Control:

- Implemented rigorous microbiological quality control measures, including the assessment of key microbial indicators such as Lactobacillus and Streptococcus thermophilus.

- Employed GRAM staining techniques for bacterial identification and conducted an antibiogram comparison to assess antibiotic susceptibility patterns.

II-2- Location and duration of study

Our experimental work was conducted at both the Microbiology Laboratory 2 and the Laboratory of Ecology and Soil Studies within the Faculty of Natural and Life Sciences at the University of Tiaret.

The study took place over a period extending from February 18th to March 3rd, 2024.

II-3- Instruments used

The materials used for the study are listed in the tables below

TABLE N°01: Equipment used

Biological materials	**Devices**	**Glassware**	**Culture mediums**
-Yogurt 8 samples of yoghurt from the two brands (Soummam; Danone) -Lactic ferments (BENEFLORE)	- Optical microscope (OPTIKA) - Micropipette - Autoclave - Icebox - Sterilizer - Analytical balance (SARTORIUS) - Incubator: **37°**C; 43°C (BINDER) - Water bath (MEMMERT) -PH meter (HANNA)	- Erlenmeyer flasks - Beakers - Graduated cylinders - Petri dishes - Desiccator - Graduated burette - Glass spreader	- MRS - M17

TABLE N°02: Material used

Thinners	**Other appliances and utensils**	**Chemicals**
-Physiological water	-Whisker -Funnel - Sterile cotton swab - Culture swab - Bunsen burner - Rack -Timer	-Distilled water -NaOH -Phenolphthalein -Disinfectant

II-4- Sampling

8 samples of yoghurt from the two brands (Soummam; Danone) of the same lot, the most consumed by the population, according to traders, were taken from points of sale of different shops. These samples were placed in a cooler and transported directly to the laboratory for physicochemical and microbiological analysis.

II-5- Experimental protocol

The following figure outlines the experimental procedures used in this study. These procedures detail the steps followed in this study.

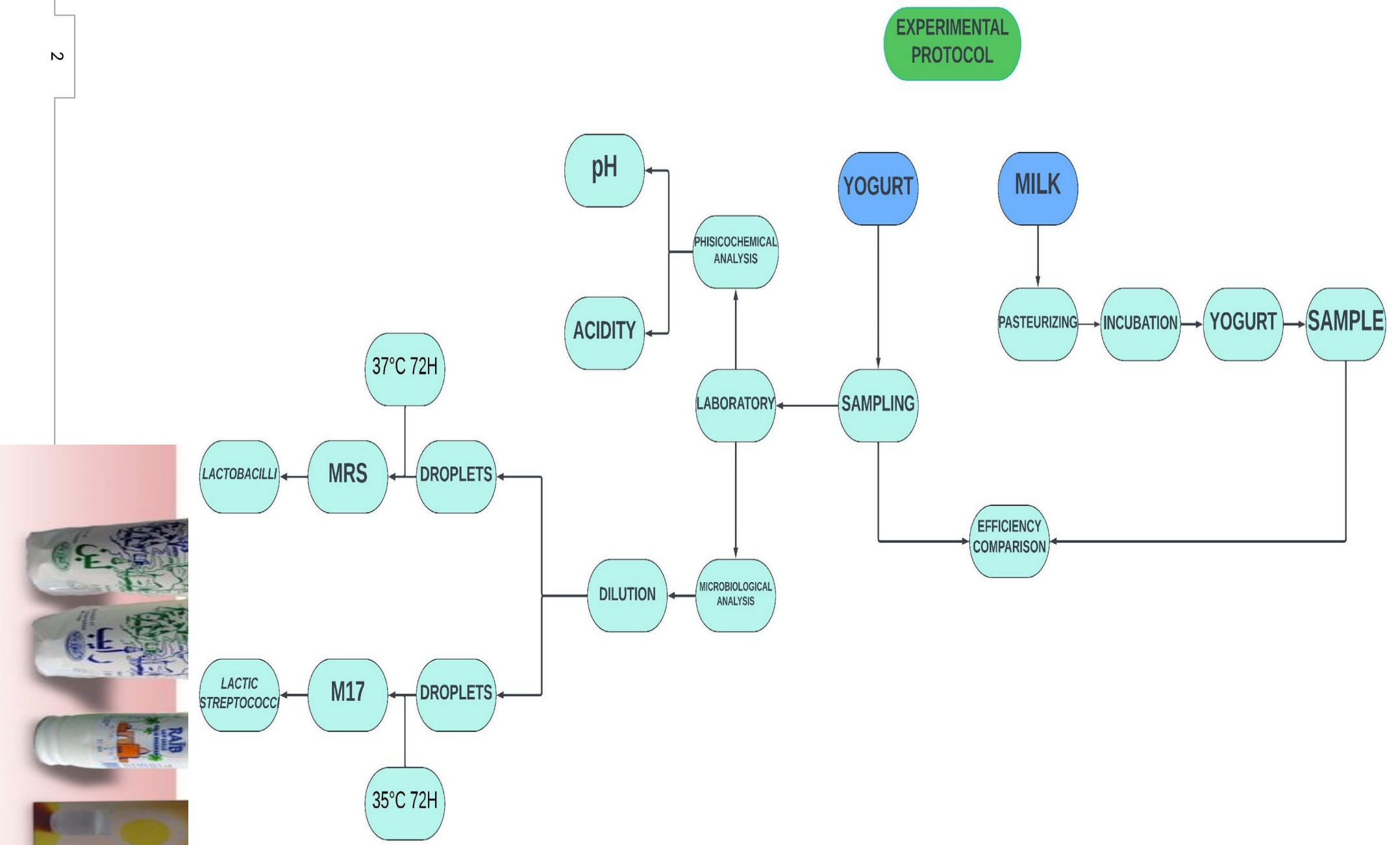


Figure N° 01: Scheme of the experimental protocol

II-5-1 Survey

We have formulated a survey in order to gauge the public's awareness about probiotic foods especially dairy products

The questions included the age and gender of the survey takers, their educational level and their occupation

Whether they consume famous brands of dairy products and how often

Instead of asking about probiotics we asked about the ''bifidus'' term as it is more known especially in the dairy products advertisements

The survey was conducted via ''google forms'' and in person

While the online survey targeted tech-savvy people the physical survey targeted the older population

The following is a copy of the conducted survey

Figure N° 02: copy of the survey

II-5-2- Analysis techniques

II-5-2-1- Physicochemical analyses

II-5-2-1-1- pH measurement

A-Definition

The relative concentration of hydrogen ions (H^+) and hydroxide ions (OH^-) in a solution (**National Institute of Standards and Technology., 2020**).

B-Principle

The principle is to measure the difference in potential between a measuring electrode and a reference electrode combined in a combination electrode system (**Lakhdar *et al.*, 2014**)

C- modus operandi

- Calibrate the pH meter with distilled water

- Thoroughly mix the contents of the yogurt container with a spatula

- Immerse the pH meter probe into the yogurt container

- Read the result displayed on the screen

II-5-2-1-2- Determination of the titratable acidity

A-Definition

Titratable acidity in yogurt refers to the quantity of lactic acid that is produced when lactose is converted into lactic acid by lactic acid bacteria. **(Tamime and Robinson, 2015)**

B- Principle

The measurement of titratable acidity is carried out by neutralizing a sample of the culture to be analyzed using sodium hydroxide (NaOH); the titration is done in the presence of phenolphthalein, a colored indicator whose endpoint is a pink coloration.

The result can be expressed according to the difference in units which are a function of the molarity of the sodium hydroxide solution used as the neutralizing alkaline agent.

The Dornic degree (°D) is the most widely used unit, one Dornic degree corresponds to 0.01% (or 0.1 g/L) of lactic acid present in the culture medium. This titration method is still widely used in the dairy industry. Yogurt acidity values are around 100°D. **(Tamime & Robinson, 2015)**

C-Modus operandi

- Take a 10 ml volume from the yogurt container using a graduated pipette

- Pour this volume into a beaker and add 2 to 3 drops of phenolphthalein

- Add the sodium hydroxide solution from the burette drop by drop until the color changes to pink

- Read the volume of sodium hydroxide solution used from the burette

D- Measurement and monitoring of acidity

- **Calculation (AOAC International, 2019)**

Acidity is given by the following expression.

$$A = V_1 .10$$

A: Titratable acidity expressed by °D

$\mathbf{V_1}$: The volume of the NaOH base (N/9) poured in ml.

II-5-2-2-Microbiological analysis

II-5-2-2-1- Objective of microbiological control

Implementing controls is essential to ensure the optimal microbiological quality of the yogurt produced. These controls serve multiple purposes, including confirming the presence of beneficial bacteria promoted by the product. By conducting rigorous microbiological assessments, such as microbial enumeration and identification, microbiological standards can be upheld, safeguarding both the safety and quality of the yogurt. Additionally, these controls provide insights into the viability and abundance of probiotic or beneficial bacteria, thereby reinforcing the product's health-promoting properties. Overall, meticulous monitoring and control measures are indispensable in maintaining the microbiological integrity and efficacy of yogurt production processes.

II-5-2-2-3- Preparation of sample and test sample

Before opening the yogurt container, it is crucial to eliminate any potential sources of contamination by thoroughly cleaning the outer surface of the container around the area from which the sample will be taken. This cleaning process can be effectively carried out using ethanol to minimize the risk of additional contamination. Once the cleaning is completed, it is essential to open the container in an aseptic manner to maintain the integrity and quality of the yogurt sample.

II-5-2-2-4- Preparation of the 10^{-1} and 10^{-3} dilutions

To conduct the microbiological analysis of the yogurt, follow these steps:

1. Shake the contents of the yogurt jar thoroughly.

2. Using a sterile spatula, take 10 grams of yogurt and introduce it into 90 ml of saline solution to achieve a 10^{-1} dilution.

3. From the 10^{-1} dilution, take a volume of 1 ml and transfer it into a test tube containing 9 ml of saline solution to create a 10^{-2} dilution.

4. Similarly, from the 10^{-2} dilution, take 1 ml and add it to another test tube containing 9 ml of saline solution to obtain a 10^{-3} dilution. (**JoVE Science Education Database, Microbiology, Serial Dilutions and Plating: Microbial Enumeration, 2024**)

II-5-2-2-5- Microbiology Research

II-5-2-2-5-A-Detection of lactobacilli

Lactobacilli are a diverse group of rod-shaped, Gram-positive, facultatively anaerobic bacteria belonging to the family Lactobacillaceae. They are recognized for their fermentative metabolism, which primarily involves converting carbohydrates such as sugars into lactic acid (**LeBlanc *et al.,* 2017**). To detect *Lactobacilli*, a sample is pipetted onto MRS agar petri dishes, with 100µl distributed evenly using a bent pipette. These plates are then placed in a desiccator and incubated at 37°C for 72 hours. Upon examination, colonies are identified as small, white, raised, and opaque.

II-5-2-2-5-B-Detection of *lactic streptococci*

Lactic streptococci constitute a diverse group of Gram-positive, cocci-shaped (spherical) bacteria within the family *Streptococcaceae*, renowned for their fermentative metabolism, primarily converting carbohydrates like sugars into lactic acid (**Leroy & Chopin, 2016**). To detect *lactic streptococci*, 100µl of each sample is pipetted onto 3 M17 agar petri dishes, ensuring even distribution with a bent pipette. The inoculated plates are then placed in a desiccator and incubated at 43°C for 72 hours. Upon inspection, colonies are identified as small, smooth, entire, and translucent.

II-5-2-2-6-Gramm test

Definition

The Gram stain is a technique utilized to categorize bacteria into Gram-positive and Gram-negative groups based on their cell wall structure. Gram-positive bacteria feature a thick peptidoglycan layer that retains the crystal violet stain, causing them to appear purple under the microscope. Conversely, Gram-negative bacteria possess a thinner peptidoglycan layer and an outer membrane that does not retain the crystal violet stain. As a result, these bacteria lose the purple color and appear pink or red after being counterstained with a dye such as safranin (**Baron *et al.*, 2019**).

Analytical technique

1. Prepare bacterial smears on a glass slide using isolated colonies from the agar plate and allow to air dry.
2. Flood the slide with crystal violet stain for 1 minute.
3. Rinse gently with distilled water.
4. Flood the slide with iodine solution for 1 minute.
5. Rinse the slide gently with distilled water.
6. Decolorize by exposing the slide to 95% ethanol for 10-20 seconds.
7. Rinse gently with distilled water.
8. Counterstain by flooding the slide with safranin for 45 seconds.
9. Rinse gently with distilled water.
10. Gently blot dry with bibulous paper or paper towel.
11. Examine the slide under a bright field microscope under oil immersion.

Reading

- Purple/blue color:

- Indicates the cells are Gram-positive bacteria.

- The thick peptidoglycan layer in their cell walls retained the crystal violet dye during the decolorization step.

- Pink/red color:

- Indicates the cells are Gram-negative bacteria.

- The thin peptidoglycan layer and outer membrane allowed the crystal violet to be washed out during decolorization.

II-5-3- Yogurt making

To make yogurt, begin by pasteurizing 2 liters of milk in a 2-liter Erlenmeyer flask by placing it in an 82°C water bath for 10 minutes. Then, take 60 mL of the milk and allow it to cool to 46°C in a 43°C water bath. Next, add 4 grams of yogurt starter to the 60 mL of milk in a graduated cylinder, whisking until evenly dissolved. Inoculate the 2 liters of milk by adding the starter mixture 1 mL at a time, gently swirling the flask after each addition. Divide the inoculated milk equally into two sterile 1-liter Erlenmeyer flasks, loosely covering each with a lid. Incubate the flasks in a 43°C incubator for 12 hours. Finally, transfer the flasks to a refrigerator for storage.at 4°C for 6 hours (**Agustinah *et al.*, 2019**)

Figures N° 03: Picture of yogurt preparation

II-5-4-Efficacy comparison

With our homemade yogurt containing known ferments as a control group and no additives or conservatives, we will proceed to evaluate and compare their antimicrobial properties.

- Analytical technique

- immerse 2 disks into Activia yogurt serum, and 2 disks into Activia yogurt supernatant.

- immerse 2 disks into Acti+ yogurt serum and 2 disks into Acti+ yogurt supernatant.

- immerse 2 disks into homemade yogurt serum, and 2 disks into homemade yogurt supernatant.

- inoculate each Petri dish with E. coli and Salmonella cultures using sterile techniques to obtain well-isolated colonies.

- use sterile spreaders to evenly distribute the cultures on the agar surface.

- deposit the following disks onto each inoculated Petri dish:

1 Activia serum disk

1 Activia supernatant disk

1 Acti+ serum disk

1 Acti+ supernatant disk

1 homemade yogurt serum disk

1 homemade yogurt supernatant disk

- incubate at 37°C for 48 hours

- **Reading**

  ·Determine the diameter of the inhibition halos surrounding each disk

  ·Compare inhibitory effects of different yogurt samples on the 2 bacteria

Results and discussion

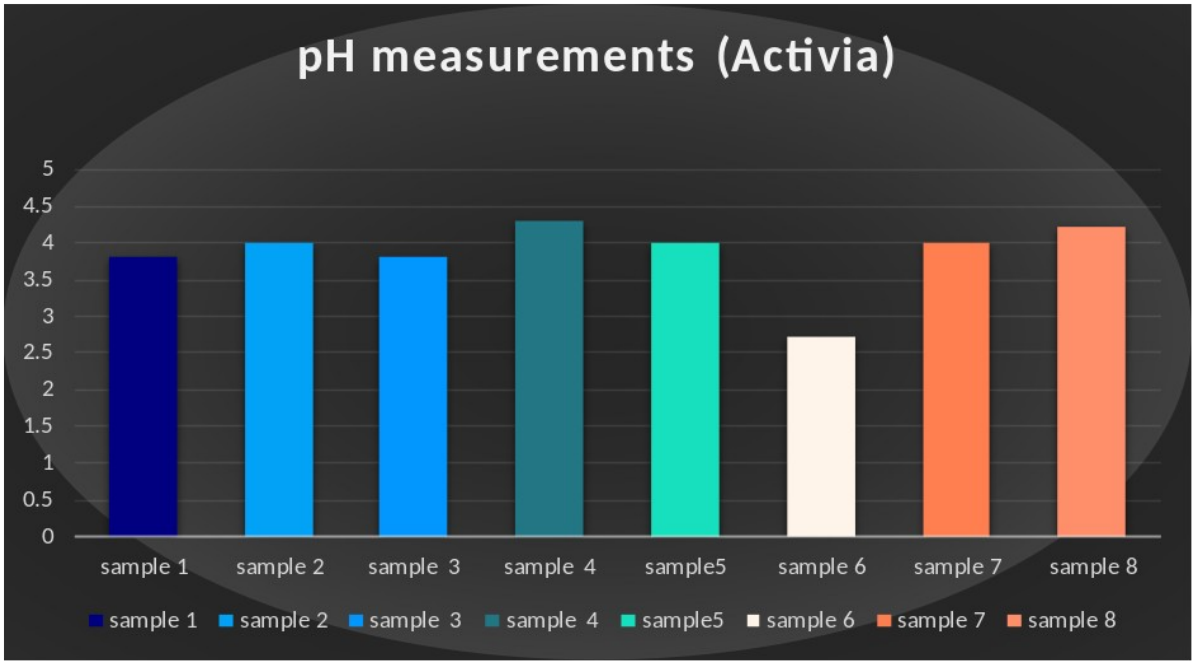
III-1-Results and discussion

III-1-1- Physicochemical analyses results

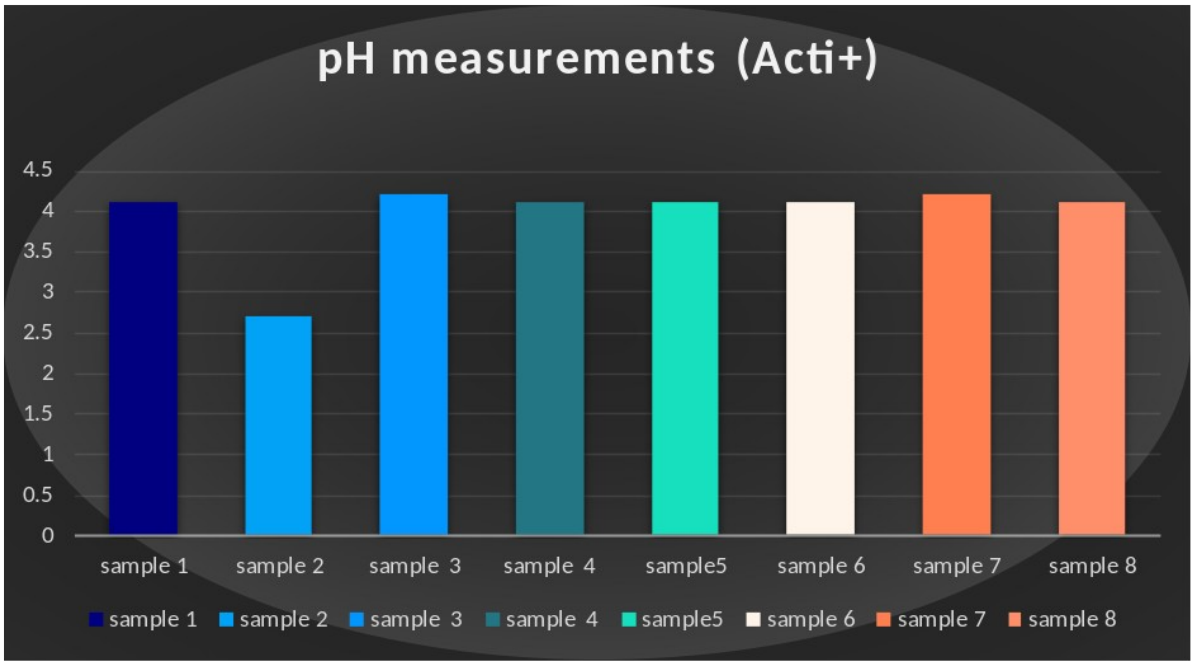
For physicochemical analyses, we took 8 units for each sample in order to get more accurate results

III-1-1-1-pH

The results obtained from our work are represented by the following figures (1, 2, 3, 4). (Refer to the corresponding tables in Annex 2)



A: Histogram of the pH analysis results from (Activia)



B: Histogram of the pH analysis results of (Acti+)

Figures N° 04 :(A, B,) Histograms provide a visual representation of the results obtained from pH analysis.

The pH value serves as a reliable indicator of yogurt freshness, primarily due to the activity of lactic acid bacteria, which leads to a decrease in pH over time. Initially,

yogurt typically exhibits a pH ranging from 4.25 to 4.50. However, as the shelf life progresses, the pH of yogurt samples gradually declines due to increased activity of lactic acid bacteria (LAB) (**JGS Ranasinghe *et al.*, 2016**).
Our analysis of collected yogurt samples confirms this trend, with pH values falling within the range of 3.6 to 4.5. This adherence to the standard pH range signifies the quality and compliance of the yogurt products.

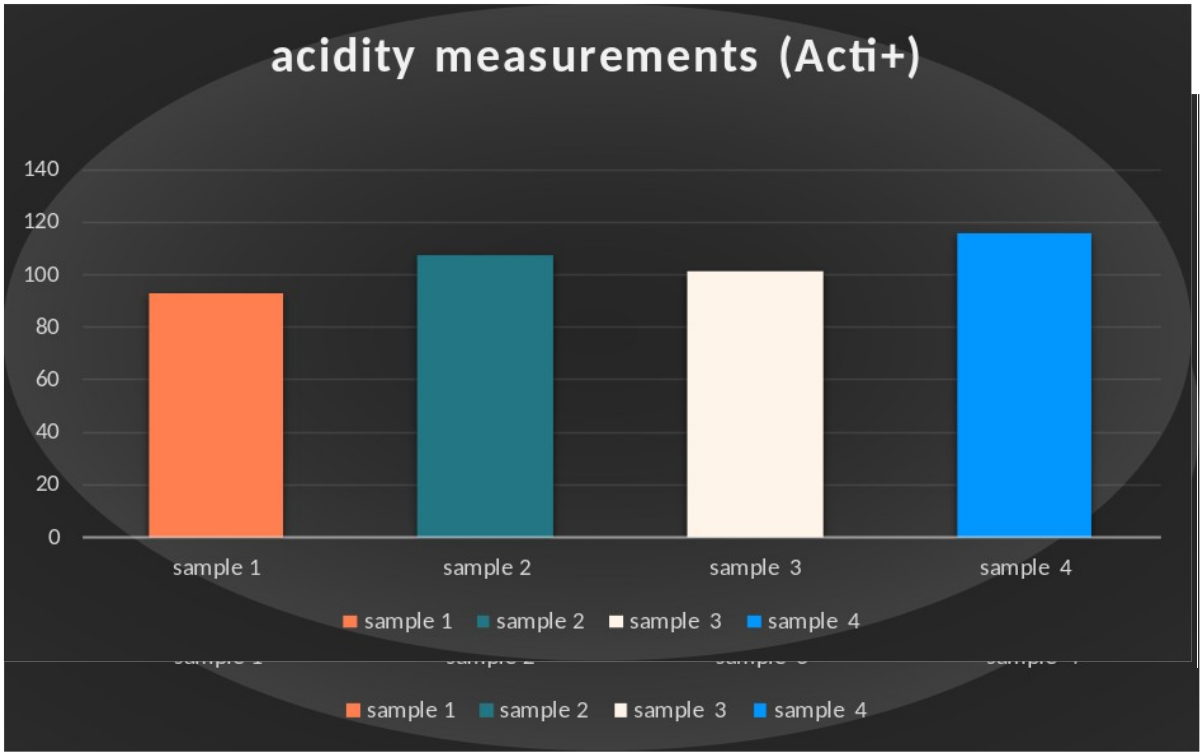
This correlation between pH and yogurt freshness underscores the importance of monitoring pH levels as part of quality assurance measures in the dairy industry.
The result underscores the pivotal role of pH as an indicator of yogurt freshness and quality. The gradual decrease in pH over time is directly linked to the activity of lactic acid bacteria (LAB), responsible for lactose fermentation and lactic acid production. This progressive acidification is a natural process contributing to the characteristic taste of yogurt and its preservation (**Burke *et al*., 2018**)

The fact that pH values of all collected yogurt samples fall within the normal range of 3.6 to 4.5 is encouraging, indicating compliance with established quality standards. It suggests that manufacturing and storage processes have been controlled, thus maintaining product quality throughout its shelf life.

These findings highlight the importance of regularly monitoring yogurt pH throughout its lifespan to ensure quality and safety for consumers. Moreover, they reinforce the notion that pH control should be integrated into quality control protocols in the dairy industry to ensure product consistency and compliance. Finally, these conclusions can serve as a basis for further research aimed at optimizing manufacturing processes and extending the shelf life of dairy products.

III-1-1-2-Acidity

The results of the acidity measurements for both brands are demonstrated in the histograms below



A: Histogram of the acidity analysis results of (Activia)

B: Histogram of the acidity analysis results of (Acti+)

Figure N°05 (A, B) Histograms provide a visual representation of the results obtained from acidity analysis.

Bioactive yogurts typically exhibit a titratable acidity falling within the range of 80°D to 120°D (**Tamime *et al.*, 2015**). The acidity values of all collected yogurt samples in our study fall within this range, indicating conformity to established standards.

The acidity of yogurt can be influenced by various factors, including the type of milk used, fermentation time, and the specific strains of lactic acid bacteria present in the starter culture. Whole milk yogurt typically demonstrates slightly lower acidity compared to yogurt made with skim milk due to the partial conversion of lactose to galactose during fermentation (**Tamime *et al.*, 2015**). This reduction in available lactose substrate limits lactic acid production, resulting in a milder acidity profile.

Additionally, fermentation time plays a significant role in determining yogurt acidity. Extended fermentation periods lead to increased acidity as lactic acid bacteria continue to convert lactose into lactic acid (**Tamime *et al.*, 2015**).

Furthermore, the choice of starter culture can impact the rate and extent of acidification. Different strains of lactic acid bacteria may exhibit varying efficiencies

in lactose conversion, affecting the final acidity of the yogurt product (**Tamime *et al.*, 2015**).

By understanding these factors, yogurt manufacturers can adjust production parameters to achieve desired acidity levels and meet quality standards consistently.

III-1-2-Microbiological analyses results

III-1-2-1-*Lactobacillli* isolation

The colony morphology of the bacteria typically observed in yogurt exhibits various characteristics. These colonies often display a fusiform or elongated shape, although round or irregular forms are also possible. Their size ranges from small to medium, with diameters typically falling within the range of 1-3 mm. Colonies generally have a flat or slightly raised elevation, with edges that appear either entire or slightly lobate. Surface texture may vary, ranging from smooth to slightly rough, while the predominant color observed is usually white or cream-colored (**Vos *et al.*, 2016**).

Regarding the analysis of the two yogurt samples, notable differences were observed. The sample obtained from Danone Activia showed the presence of lactobacilli, indicating the presence of this specific bacterial strain. Conversely, the sample from Sommame Acti+ did not exhibit detectable levels of lactobacilli, suggesting the absence of this strain in the product.

These findings underscore the importance of microbial analysis in yogurt quality control and highlight the variability that can exist between different yogurt products.

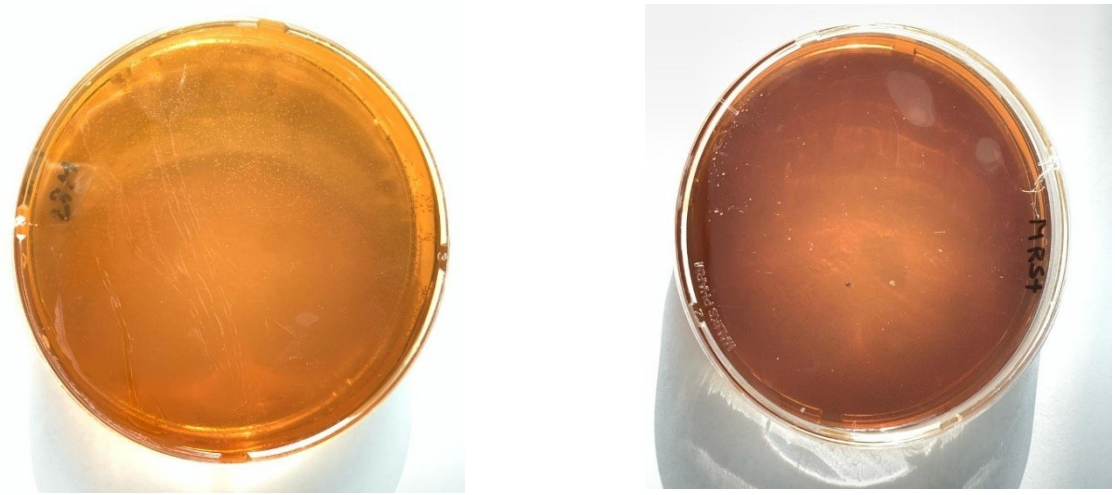


Figure N°06: Results of *Lactobacilli* incubation on MRS culture medium from (Activia and Acti+) (left,right)

III-1-2-2-*Lactic streptococci* isolation

The colony morphology of the bacteria typically observed in yogurt exhibits various characteristics while circular colonies are possible, these bacteria frequently adopt elongated or even spindle-shaped forms. In some instances, irregular colonies may also be observed. The size of these colonies varies, typically ranging from small to medium, with diameters falling between 1 and 3 millimeters. Generally, lactic streptococci colonies have a flat or slightly raised profile, and their edges may appear smooth or with slight lobations. The surface texture can vary from smooth to slightly rough, while the predominant color is typically white or cream-colored. (**Vos *et al.*, 2016**).

Regarding the analysis of the two yogurt samples, no differences were observed. Both samples showed the presence of *Lactic streptococci*, indicating the presence of this specific bacterial strain.

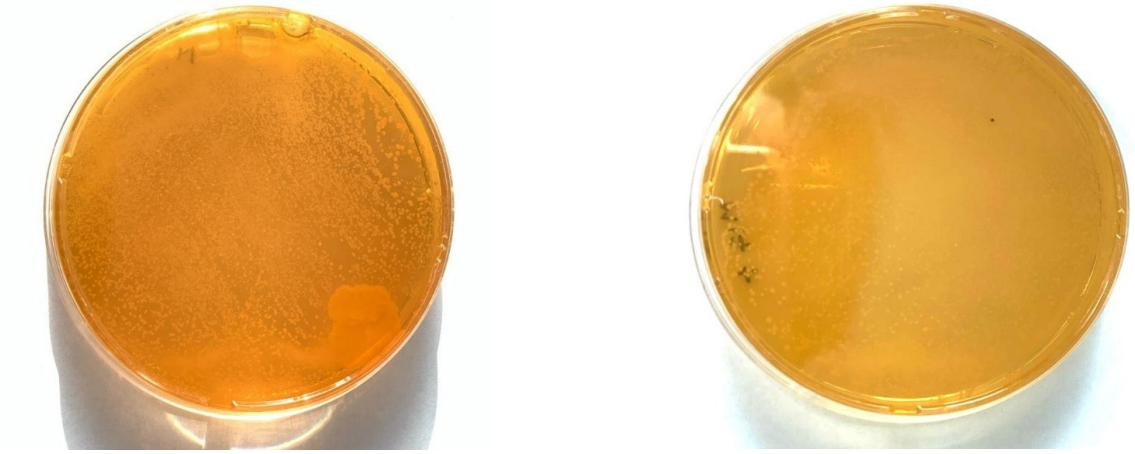


Figure N°07 : Results of *Lactic streptococci* incubation on M17 culture medium from (Activia and Acti+) (left,right)

III-1-2-3-Gram stain test

Interpreting the results of a Gram stain involves observing the color of the bacterial cells under the microscope after staining. Cells that appear purple or blue are classified as Gram-positive bacteria due to their thick peptidoglycan layer retaining the crystal violet dye. In contrast, cells that appear pink or red are Gram-negative bacteria, as their thinner peptidoglycan layer and outer membrane allow the crystal violet to be washed out, taking up only the counterstain. **(Baron *et al.*, 2019)**

The Gram staining revealed the following results:

The interpretation of Gram staining results provides valuable insights into the microbial composition of samples. In Gram staining, the color of bacterial cells observed under the microscope after staining indicates their Gram classification.

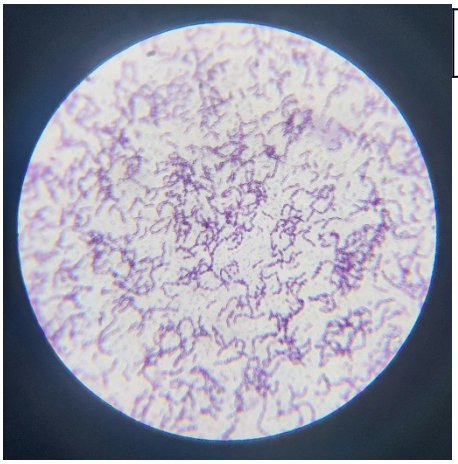
In this context, purple or blue-colored cells indicate Gram-positive bacteria, characterized by a thick peptidoglycan layer that retains the crystal violet dye. On the other hand, pink or red-colored cells are indicative of Gram-negative bacteria, possessing a thinner peptidoglycan layer and an outer membrane that allows the crystal violet dye to be washed out, with the cells taking up only the counterstain.

Based on the Gram staining results provided:

- The MRS agar sample from Danone displays chains of purple thin rods or bacilli. This observation confirms the presence of lactobacilli, which are Gram-positive bacteria.

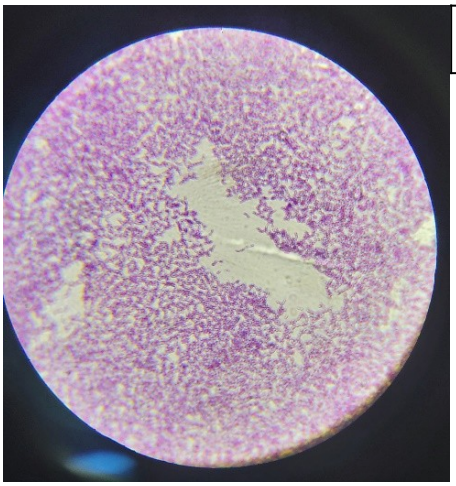
- The M17 agar samples from both Danone and Soummam exhibit pairs or small chains of purple oval-shaped cells, indicating the presence of streptococci, which are also Gram-positive bacteria.

These results align with the typical Gram staining characteristics of lactobacilli and streptococci, providing valuable information about the microbial composition of the samples from both Danone and Soummam.



Lactobacilli

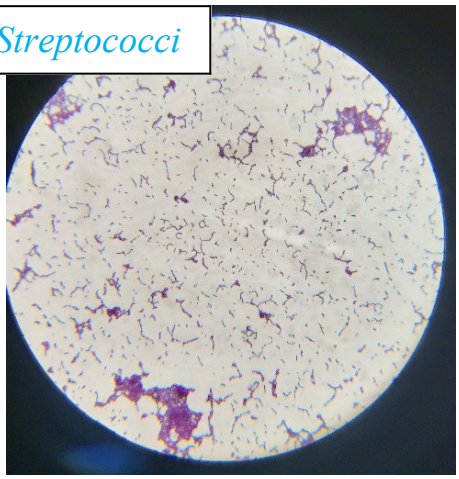
This Gram stain obtained from (40X) magnification reveals rod-shaped bacteria stained purple, indicating they are Gram-positive. The rod-shape, a defining characteristic of Lactobacillus.



Streptococci

This Gram stain obtained from (40X) magnification reveals purple cocci (spherical bacteria) arranged in chains. This chain-like morphology is a hallmark of Streptococci.

Streptococci



This Gram stain obtained from (100X) oil immersion magnification further showcases the purple Streptococci cocci arranged in chains.

Figure N°08 : Results of Gram staining

III-1-3-Yogurt efficacy comparison

III-1-3-1-Yogurt making

Obtaining yogurt fermented solely with *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Bifidobacterium lactis*, *Bifidobacterium longum* and *Streptococcus thermophilus*, without any added flavors or additives, offers a pure and traditional yogurt experience.

Lactobacillus acidophilus contributes to the characteristic tangy flavor and provides potential health benefits, such as aiding digestion and promoting gut health. Lactobacillus bulgaricus works synergistically with Streptococcus thermophilus to ferment lactose into lactic acid, resulting in the acidic taste and creamy texture typical of yogurt.

This simple formulation aligns with the traditional method of yogurt production, allowing consumers to enjoy the natural flavors and probiotic benefits of these bacterial strains without any additional ingredients. Additionally, it provides an ideal base for individuals seeking a clean, unadulterated yogurt option.

This type of yogurt may appeal to purists who appreciate the simplicity and authenticity of traditional yogurt-making techniques, as well as those who prefer to avoid added sugars or artificial flavors.

Figure N°09 : Homemade Yogurt Culture

III-1-3-2-Amtibiogram test

A procedure used to determine the effectiveness of different antibiotics against a specific bacterial isolate based inhibition zone

This method revealed the following results:

TABLE N°03: Yogurt samples and their inhibition zones

	Escherichia coli	***Staphylococcus aureus***
Danone supernatant	10	14
Danone serum	7	11
Soummam supernatant	13	14
Soummam serum	9	12
Homemade supernatant	35	31
Homemade serum	32	30

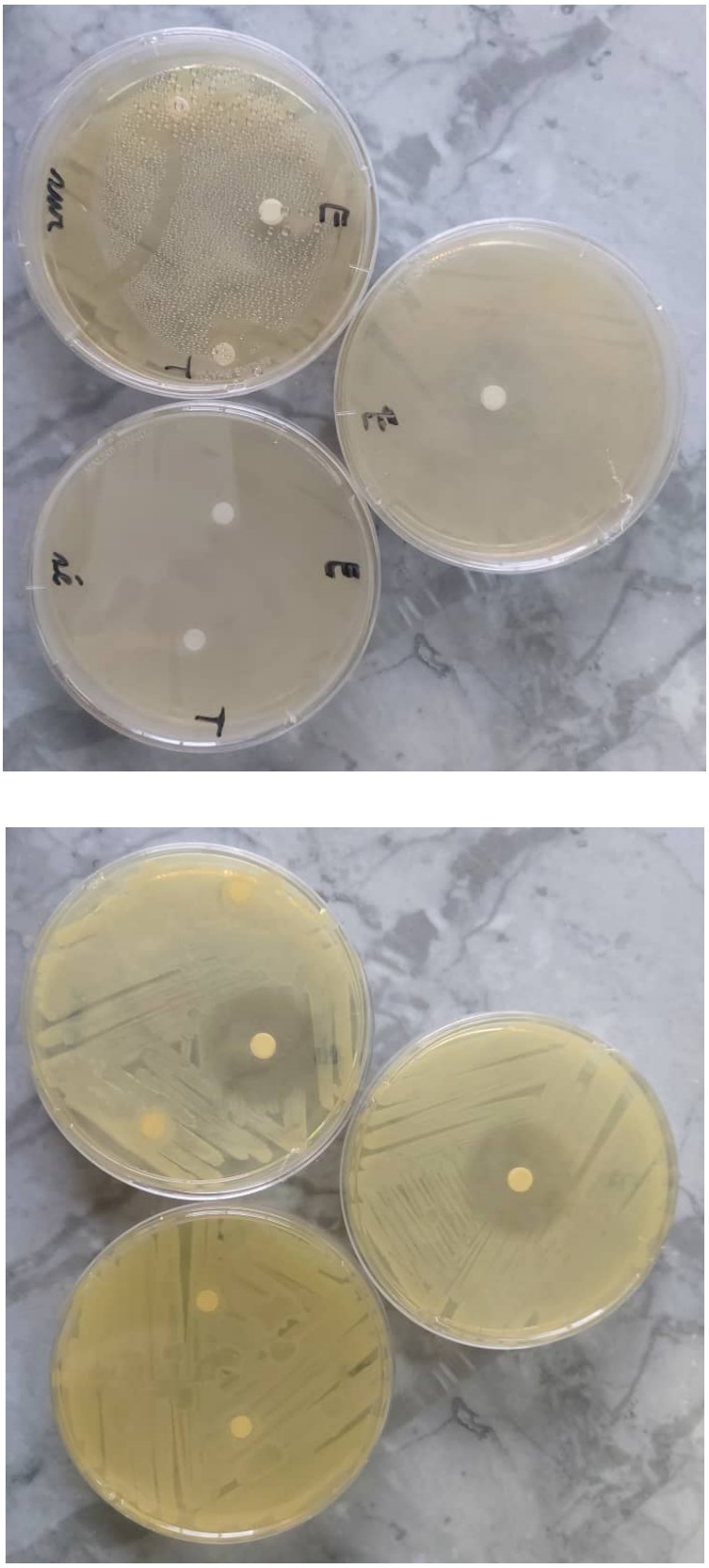


Figure N°10 : Antibiogram test of (Activia, Acti+ and homemade) samples in *E.coli* culture

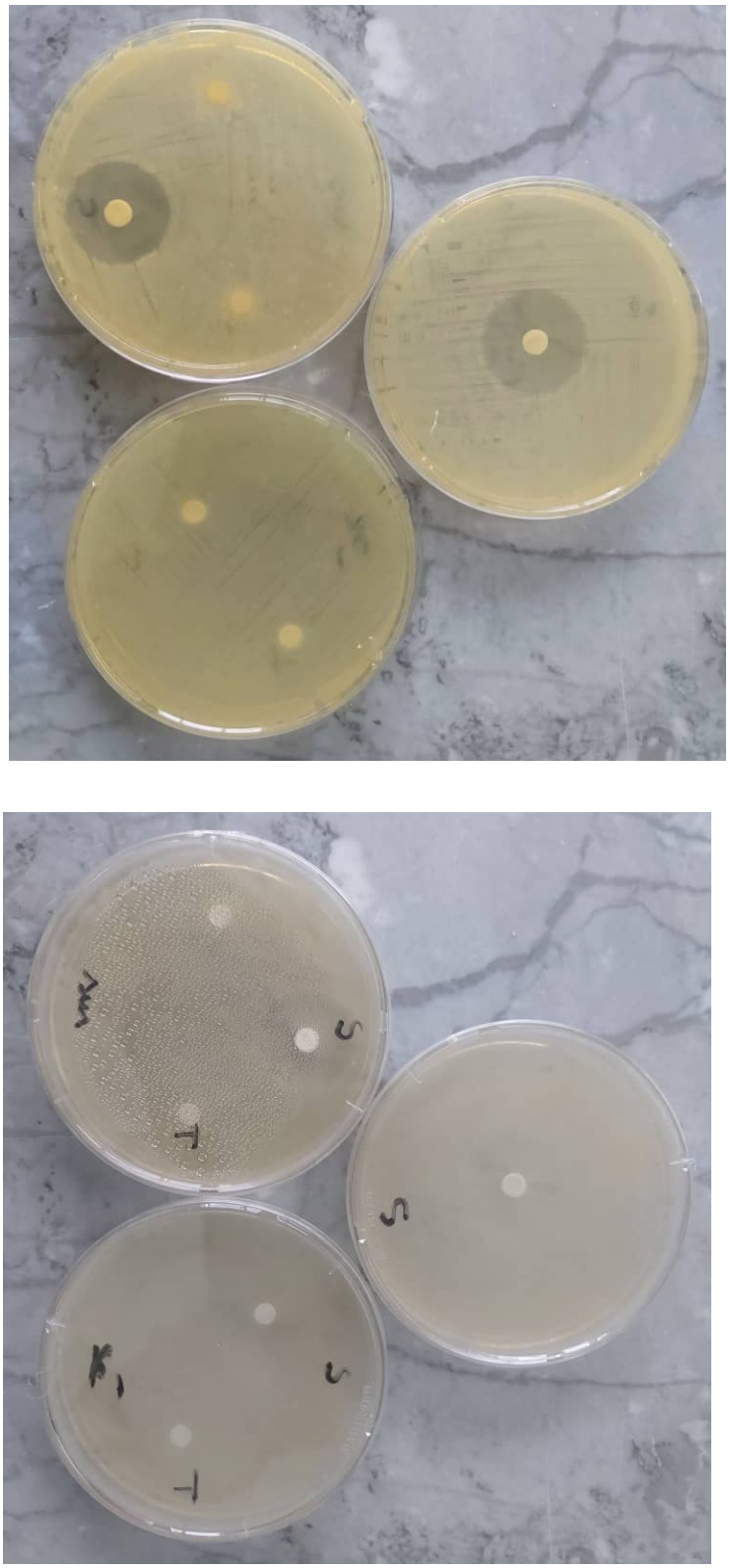


Figure N°11 : Antibiogram test of (Activia, Acti+ and homemade yogurt) samples in *Staphylococcus aureus* culture

III-1-4-Survey results

This survey aims to assess the familiarity and knowledge about probiotics in dairy products among consumers.

The survey sample comprised of [172] respondents, [70.93%] of whom are women and [29.07] are men, their ages ranging from [18-45<]

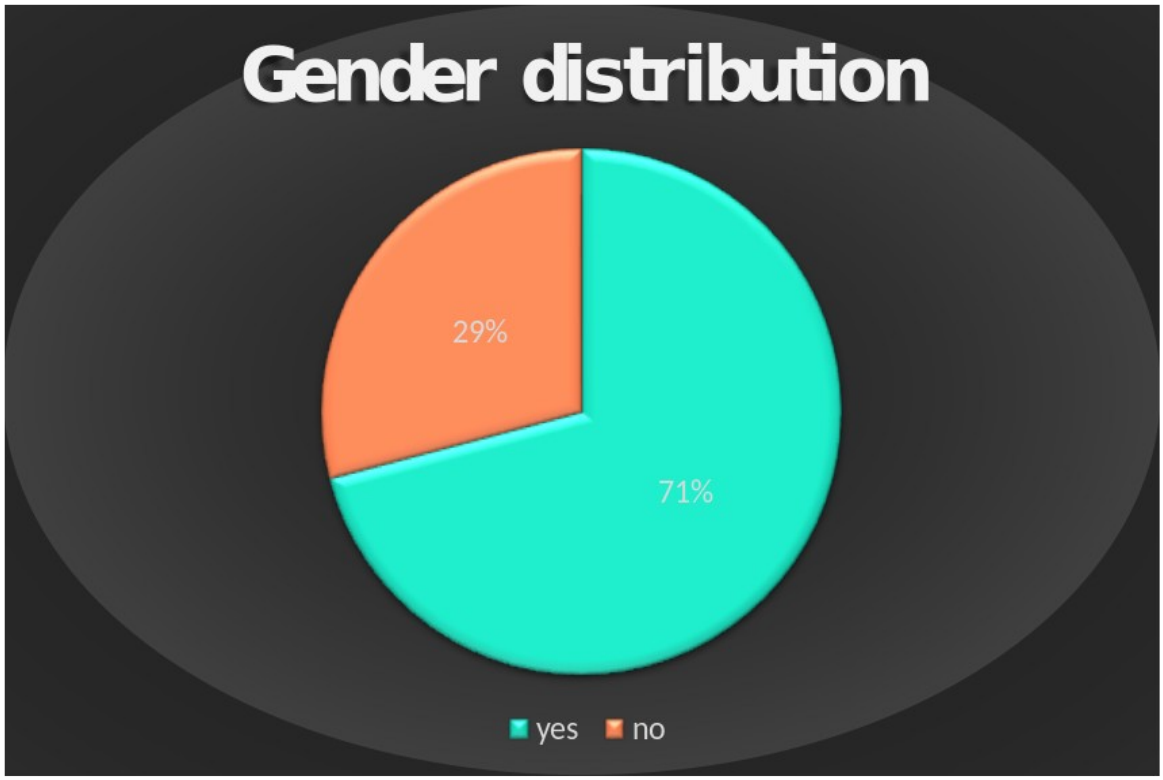


Figure N°12: Gender distribution pie chart

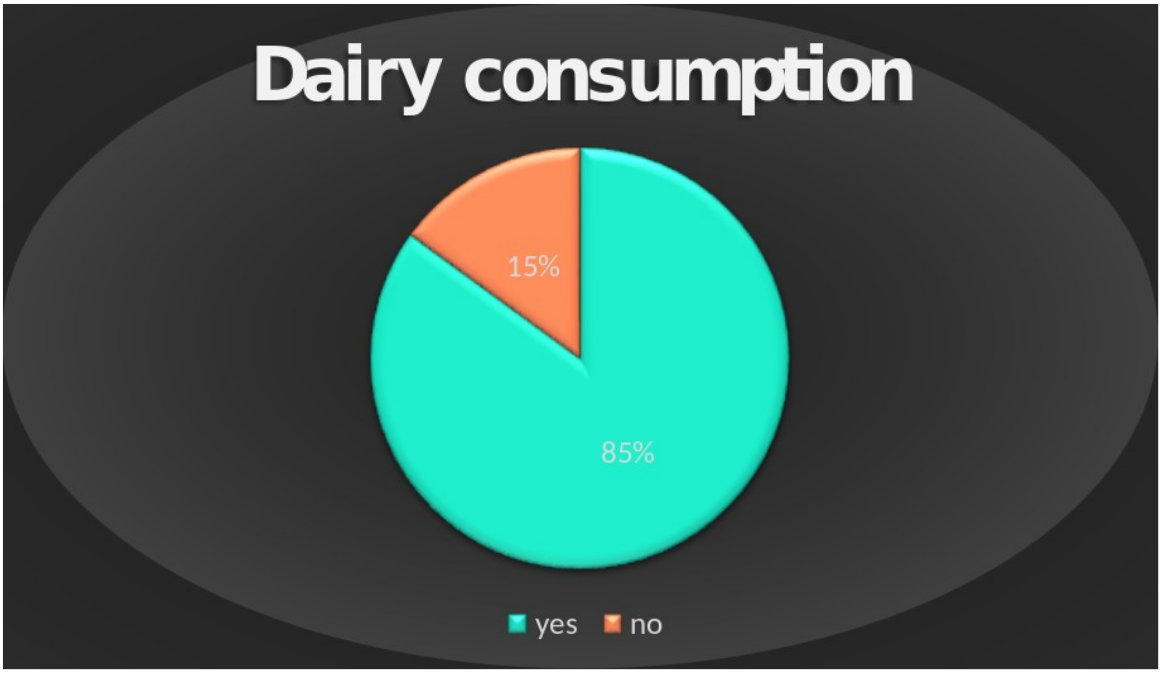


Figure N°013: Dairy consumption pie chart

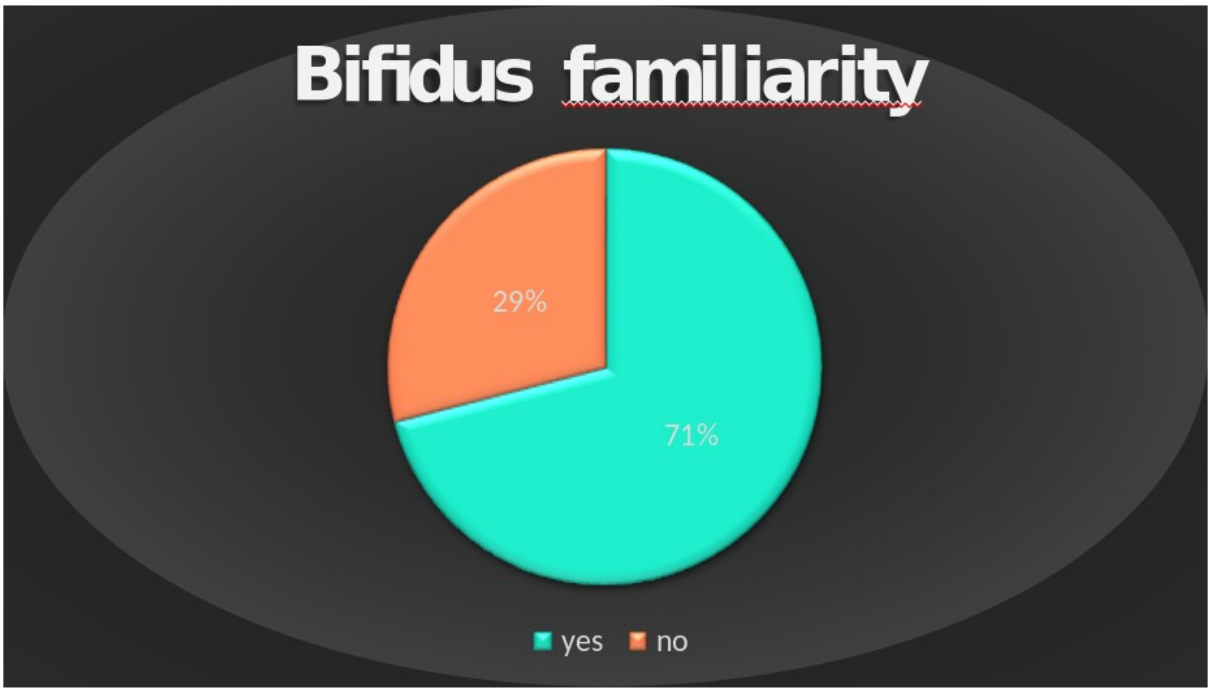


Figure N°014: Bifidus familiarity pie chart

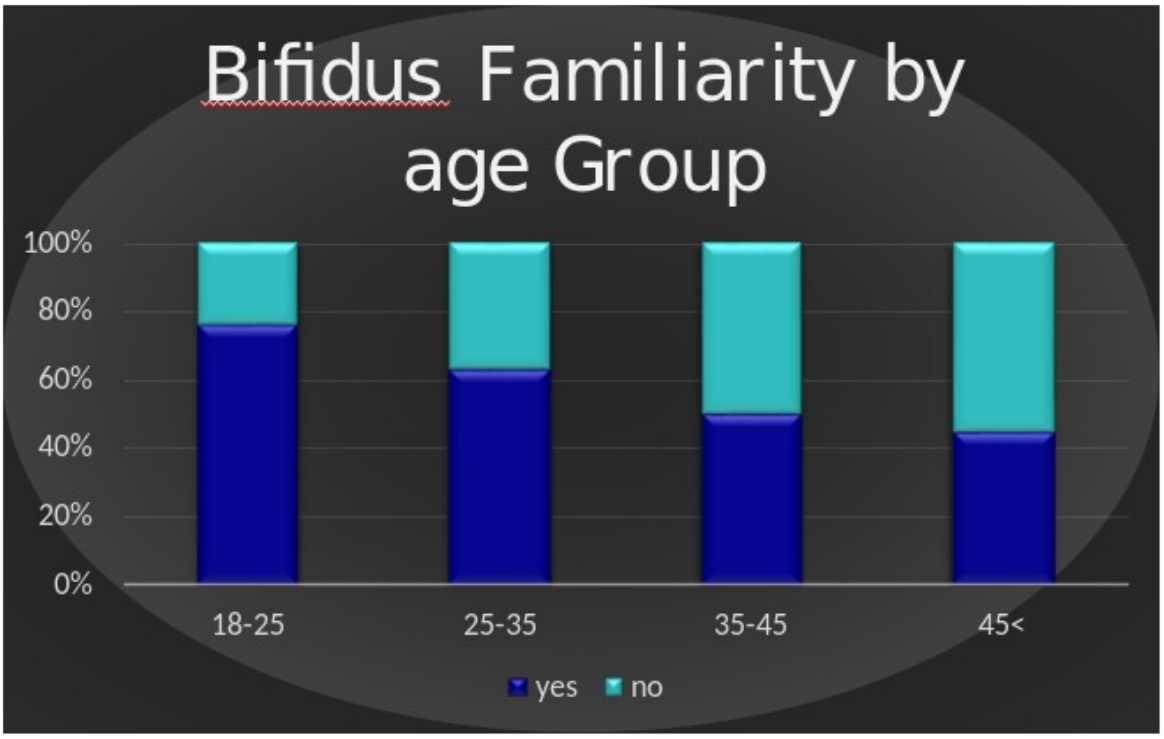


Figure N°015: Bifidus familiarity by age group bar chart

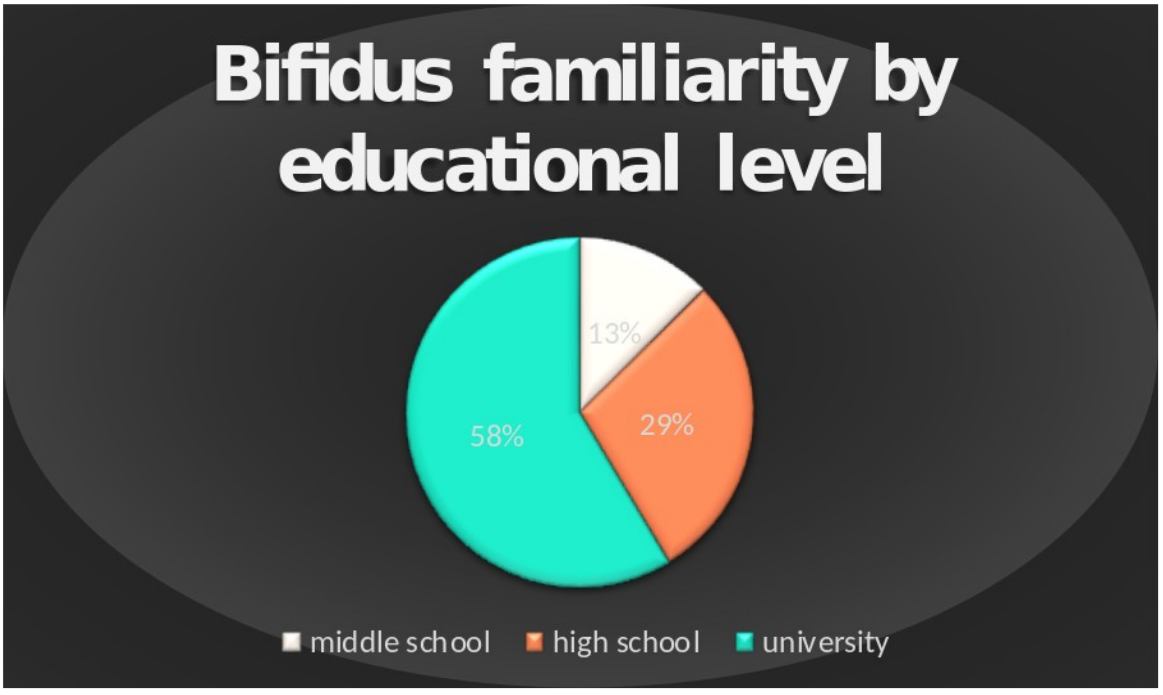


Figure N°16: Bifidus familiarity by educational level pie chart

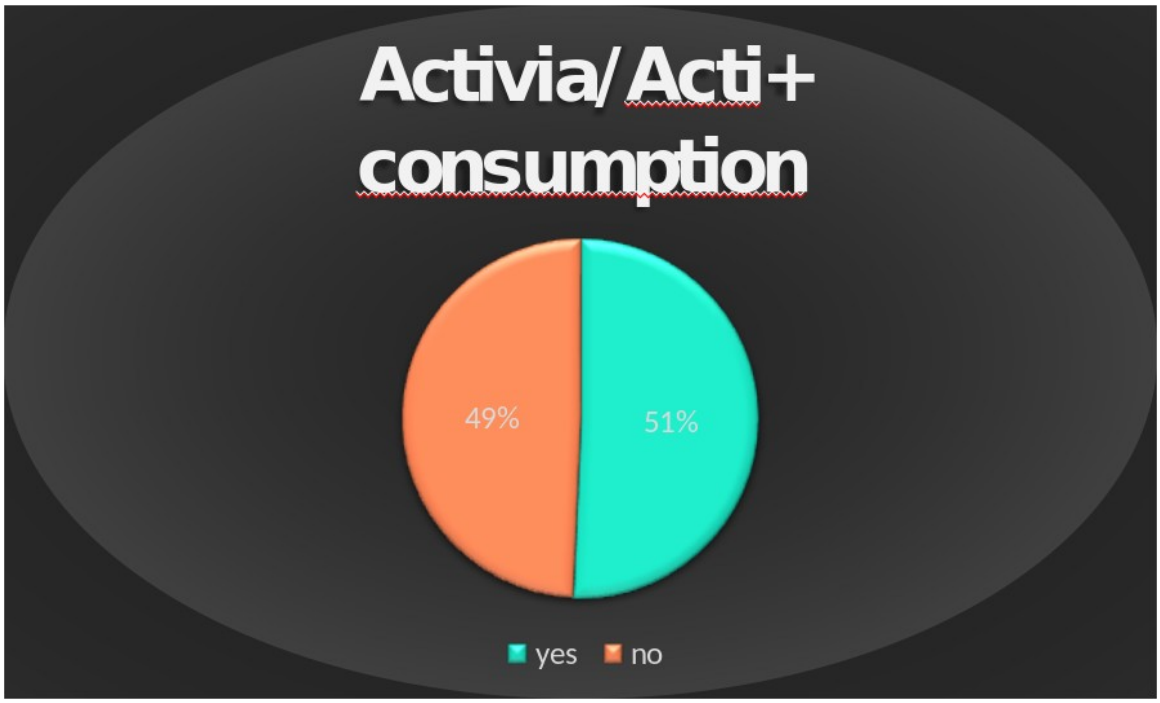


Figure N°17: Activia/Acti+ consumption pie chart

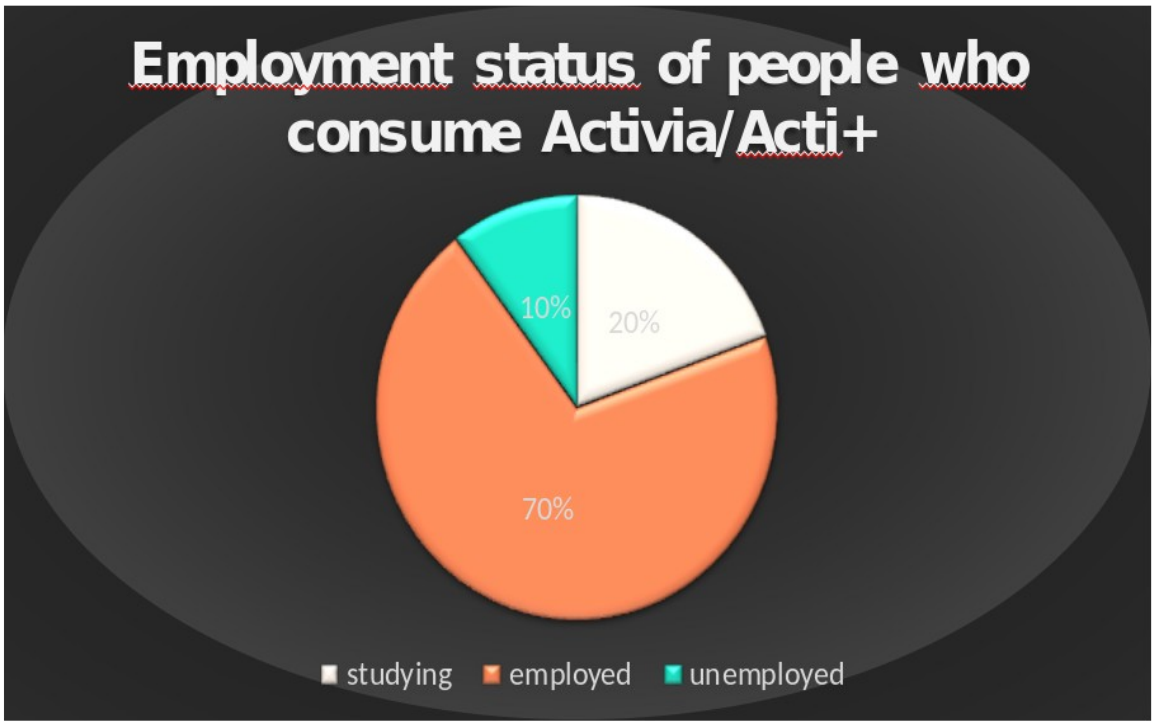


Figure N°18: Employement status of people who consume Activia/Acti+ pie chart

Table N°04: Desired health effect by age group

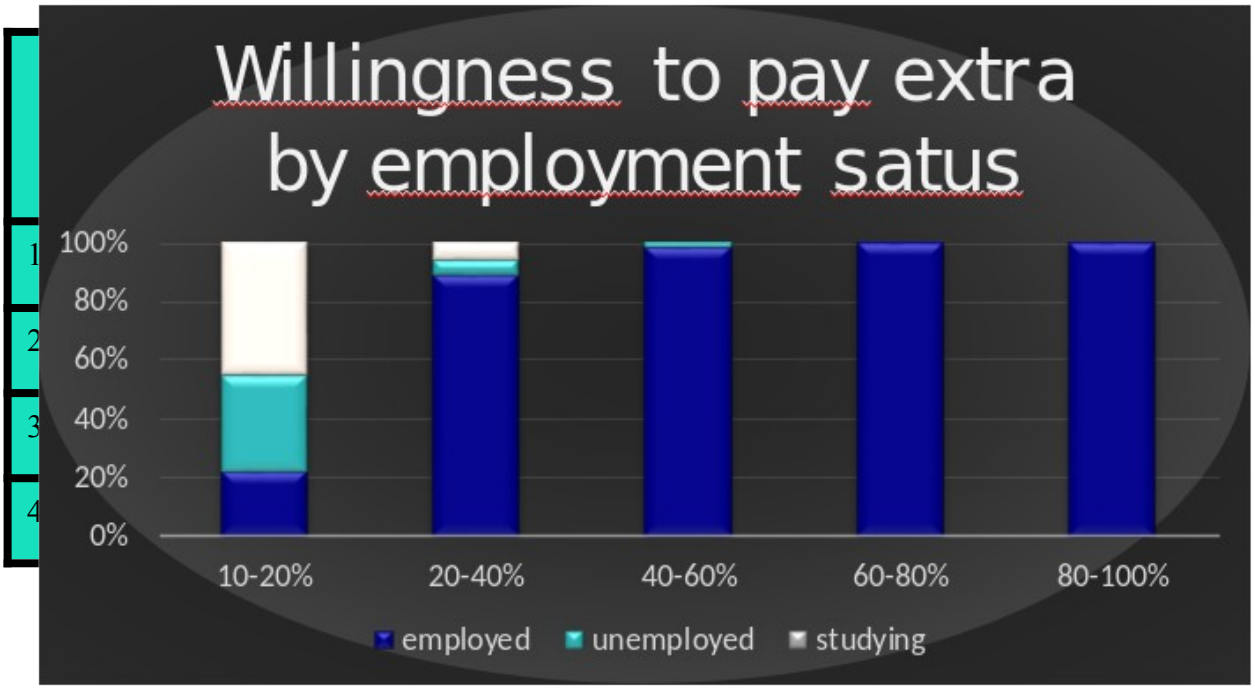


Figure N°19: Willingness to pay extra by employement status bar chart

IV-Survey discussion

Some noticeable findings are

-Out of the 172 who answered the survey the majority of the survey takers were female representing [70.93%] and males representing [29.07%]

-The majority of the survey takers consume dairy products representing [85%]

-The majority of the survey takers are familiar with the term ‘’Bifidus’’ representing [71%]

And the majority of the first age group representing ages [18-25] are familiar with the term, the same is for the second age group representing ages [25-35]. However less than half of the third age group representing ages [35-45] are familiar with the term, while very few of the fourth age group representing ages [45<] are familiar with the term

-The familiarity based on educational level was as expected with the maojority of university studentsrepresenting approximately [60%] were familiar with the term, while highschool graduates represented [30%] of all the people who were familiar with the term and middle school graduates being the minority with[10%]

-Slightly more than half of the survey takers consume probiotic products and of that half [70%] are employed and [20%] are students while [10%] are unemployed

-When asked what health benefits they want from their food, we noticed varying responses based on age group:

Most of G1 opted for better digestion followed by weight loss, G2 was the same, most of G3 opted for better digestion followed by lowering blood sugar level and blood pressure; and G4 was the same

-when asked about how much more were willing to pay for said health effects, the responses didn't differ much from expectations:

most of the survey respondents who picked the first option of a [10–20%] increase were students, as were most of the unemployed and a small portion of the employed.

The vast majority of the survey respondents who picked the second option of a [20–40%] increase were employed, followed by a small portion of students, and a smaller portion of unemployed

Almost all of the survey respondents who picked the third option of a [40–60%] increase were employed, with the exception of a very few who were unemployed

All of the survey takers that picked the fourth and fifth options of a [60–80%] and [80–100%] increase, respectively, were all employed

CONCLUSION

The objective of this study was to obtain pertinent information on a few yogurt brands widely consumed among the local population and also analyze the physicochemical and microbiological properties of these brands. Thus, based on the detailed analyses conducted, we can confidently conclude that all yogurt samples have passed the measured range of pH and titratable acidity in the Algerian official journal. Therefore, manufacturers maintain the necessary quality control

Finally, the microbiological investigations, which comprised the isolation and identification of the lactic acid bacteria, have led to interesting results. Although in both samples, Danone Activia and Soummam Acti+ lactic streptococci were found, the latter lacked detectable levels of lactobacilli. These findings indicate possible differences in the microbial composition of the products that may influence their organoleptic properties as well as their nutritional value.

Thus, the comparison of the efficacy of commercially produced and homemade yogurts has demonstrated the potential advantages of minimal processing and the absence of additives in the manufacturing of dairy products. The homemade product demonstrated excellent antimicrobial activity against the pathogenic bacteria E. coli and Staphylococcus aureus compared to the commercial samples. This proves the possible advantage of simplicity in both ingredients and the production process to produce better bioactive foods.

In conclusion, the consumer survey showed a good awareness of probiotics and functional dairy products in the population. Nevertheless, this information suggests that even more education and popularization must be conducted to inform the elderly and those with a low level of education. This survey has served the purpose of gaining valuable knowledge on the yogurt market and consumption in the Wilaya of Tiaret.

Future research could explore the potential health effects of probiotic strains found in other fermented dairy products.
Furthermore, research into what determines consumer choices and purchases could help create suitable marketing strategies to help more individuals benefit from functional dairy products.

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Appendices N°01

1- Culture media and reagents used

According to (Lewis, 2012)

❖ MRS (De Man, Rogosa, Sharpe) Medium Composition

- Component.....Amount
- Proteose Peptone .....10.0 g/L
- Beef Extract .....10.0 g/L
- Yeast Extract .....5.0 g/L
- Dextrose .....20.0 g/L
- Polysorbate 80 .....1.0 g/L
- Ammonium Citrate .....2.0 g/L
- Sodium Acetate .....5.0 g/L
- Magnesium Sulfate .....0.1 g/L
- Manganese Sulfate .....0.05 g/L
- Dipotassium Phosphate .....2.0 g/L
- Agar .....12.0 g/L
- pH ……..6.5

❖ M17 medium composition

- Pancreatic digest of casein.....5.0 g/L
- Soy Peptone.....5.0 g/L
- Beef extract.....5.0 g/L
- Yeast extract.....2.5 g/L
- Ascorbic acid.....0.5 g/L

- Magnesium sulfate.....0.25 g/L
- Disodium-β-glycerophosphate.....19.0 g/L
- Agar .....11.0 g/L
- pH …….6.5

Dilutants

- **physiological serum and distilled water**
- physiological serum ……..……...500ml
- distilled water …….1000ml

Appendices N°02

Summary table of physicochemical test results

Table 1: pH measurements (Activia)

Samples	pH	Samples	pH
1A	3.8	3A	3.9
1B	3.9	3B	3.9
1C	3.7	3C	3.8
2A	4	4A	4.2
2B	3.9	4B	4.5
2C	4.3	4C	4.2

Table 2: pH measurements (Activia)

Samples	pH	Samples	pH
5A	4.2	7A	4.1
5B	3.9	7B	4.3
5C	4	7C	3.7
6A	0	8A	4.5
6B	4.4	8B	4
6C	3.7	8C	4.2

Table 3: pH measurements (Acti+)

Samples	pH	Samples	pH
1A	4.2	3A	4.4
1B	4	3B	4
1C	4.3	3C	3.8
2A	4.1	4A	4.1
2B	0	4B	4.2
2C	4.2	4C	4.2

Table 4: pH measurements (Acti+)

Samples	pH	Samples	pH
5A	4.2	7A	4.2
5B	4.5	7B	4.1
5C	4	7C	4.3
6A	4.1	8A	4
6B	4.4	8B	4.2
6C	3.9	8C	4.1

Table 5: Acidity measurements (Activia)

Samples	Acidity	Samples	Acidity
1A	82	3A	87
1B	87	3B	115
1C	82	3C	114
2A	120	4A	69
2B	100	4B	99
2C	99	4C	108

Table 6: Acidity measurements (Acti+)

Samples	Acidity	Samples	Acidity
1A	84	3A	115
1B	85	3B	85
1C	110	3C	103
2A	108	4A	120
2B	94	4B	119
2C	119	4C	108

Appendices N°03

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ANNEXE II

SPECIFICATIONS TECHNIQUES DES TYPES DE LAIT FERMENTE

Spécifications techniques	Produits	
	Lait fermenté	Yaourt
Protéine du lait [a] (% masse/masse)	Minimum 2,7 %	Minimum 2,7 %
Matière grasse laitière (% masse/masse)	Inférieure à 10 %	Inférieure à 15 %
Acidité titrable, exprimée en pourcentage d'acide lactique (% masse/masse)	Minimum 0,3 %	Minimum 0,7 %
Somme des micro-organismes spécifiques cités à l'article 3 ci-dessus (ufc/g, au total)	Minimum 10^7	Minimum 10^7
Micro-organismes étiquetés[b] (ufc/g, au total)	Minimum 10^6	Minimum 10^6
La teneur en matière sèche laitière non grasse (% masse/masse)	Minimum 8,2 %	Minimum 8,2 %

Appendices **N°04**

Summary table of survey results

Gender distribution of survey takers

SURVEY TAKERS		
MALE	FEMALE	
50	122	TOTAL=172

Dairy consumption habits of survey takers

DAIRY CONSUMPTION		
YES	NO	
146	26	

Bifidus familiarity among survey takers

BIFIDUS FAMILIARITY		
YES	NO	
122	50	TOTAL= 172

Bifidus familiarity by age group

Age group familiarity	18-25	25-35	35-45	45<
YES	60	25	16	10
NO	18	14	16	13
TOTAL	78	39	32	23

Bifidus familiarity by educational level

MIDDLE SCHOOL	16
HIGH SCHOOL	35
UNIVERSITY	71
TOTAL	122

Activia/Acti+ consumption

ACTIVIA/ACTI+ CONSUMPTION+	
ACTIVIA	62
ACTI+	61
TOTAL	123

Employment status of people who consume (Activia/Acti+)

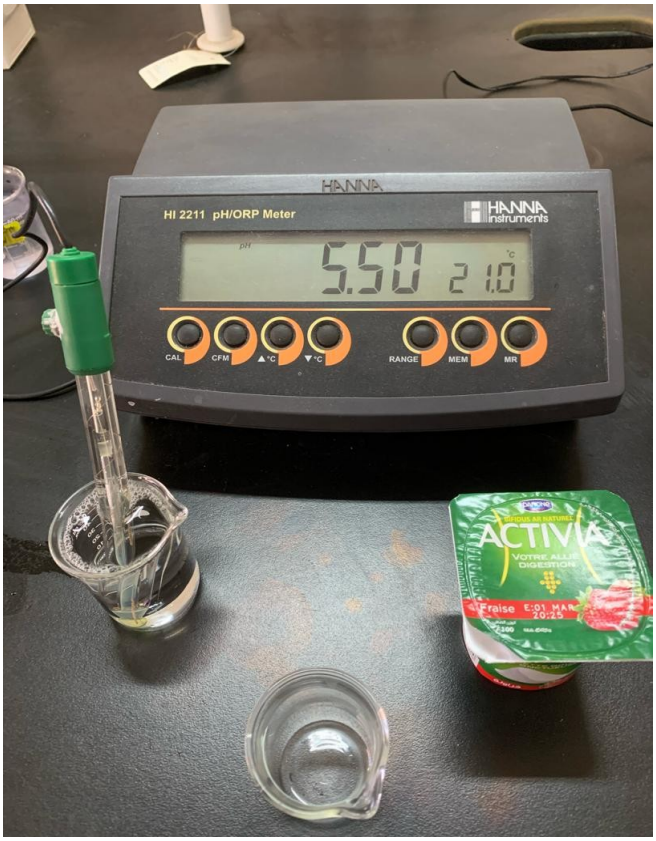
EMPLOYEMENT STATUS	
STUDYING	24
EMPLOYED	86
UNEMPLOYED	13
TOTAL	123

Willingness to pay extra by employment status

Extra pay Employment	10%-20%	20%-40%	40%-60%	60%-80%	80%-100%
STUDYING	15	7	0	0	0
EMPLOYED	8	105	17	1	1
UNEMPLOYED	11	6	1	0	0

Appendices **N°05**

Equipment utilized for the study



pH meter

Titratable acidity apparatus

Microscope

Dessiccator

Appendices N°06

Some yogurt samples

Abstract

The work consisted of studying the most consumed yogurts by the local population according to our survey and their physicochemical and microbiological characteristics. Through this work, we wished to assess the food security and quality of these dairy products. We performed physicochemical tests: pH, titratable acidity, and microbiological analyses: isolation of lactobacilli, lactic streptococci, and Gram coloration. We studied two commercial brands of yogurt, Danone Activia and Soummam Acti+, and we made our own homemade yogurt. Our homemade yogurt was fermented with known strains of LAB without any additives or preservatives.

In summary, the findings of the study indicated that all yogurt variations' pH and acidity were within the acceptable limits of the set standards. Nevertheless, the differences were noted in the microbial colony, as Soummam Acti+ had no observable lactobacilli colonies. Additionally, our homemade yogurt had the best bioactive effect against pathogenic bacteria due to the lack of additives and preservatives and the relatively simple method of preparation.

Key words: yogurt, physicochemical characteristics, microbiological characteristics, consumer, Danone, Soummam.

ملخص

هذا العمل يتضمن دراسة أنواع الياغورت الأكثر استهلاكا من قبل السكان المحليين وفقًا لاستطلاعنا وخصائصها الفيزيوكيميائية والميكروبيولوجية. ومن خلال هذا العمل، أردنا تقييم الأمن الغذائي وجودة هذه المنتجات الألبانية. قمنا بإجراء اختبارات فيزيوكيميائية: الأس الهيدروجيني والحموضة القابلة للتيطير، وكذلك تحاليل ميكروبيولوجية: عزل العصيات اللبنية والمكورات اللبنية وتلوين جرام. درسنا نوعين تجاريين من الياغورت، وهما دانون أكتيفيا وسومام أكتي + ، وصنعنا ياغورت منزلي خاص بنا. تم تخمير الياغورت المصنوع منزليًا بسلالات معروفة من البكتيريا الحمضية اللبنية بدون أي إضافات أو مواد حافظة.

باختصار، أشارت نتائج الدراسة إلى أن قيمة الأس الهيدروجيني والحموضة لجميع أنواع الياغورت كانت ضمن الحدود المقبولة للمعايير المحددة. ومع ذلك، لوحظت اختلافات في المستعمرات الميكروبية، حيث لم يُشاهد وجود مستعمرات للعصيات اللبنية في سومام أكتي +. بالإضافة إلى ذلك، كان للياغورت المصنوع منزليًا أفضل تأثير حيوي ضد البكتيريا المسببة للأمراض بسبب خلوه من الإضافات والمواد الحافظة وطريقة التحضير البسيطة نسبيًا.

الكلمات المفتاح: الياغورت، الخصائص الفيزيوكيميائية، الخصائص الميكروبيولوجية، المستهلك، دانون، صومام.