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Comparative analysis of physicochemical and microbiological characteristics in selected cheese varieties

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Abstract

This study investigated the physicochemical and microbiological status of three cheese types the most consumed in Guelma City which are cottage cheese (CC), analogue cheese (AC), and pasteurized processed cheese (PPC), by determining the chemical composition (moisture, ash, and fat), the physicochemical properties (pH and acidity), and the microbiological quality (*Staphylococcus aureus, Escherichia coli, Salmonella,* and lactic acid bacteria (LAB)). The results showed that the CC had the highest moisture content and the lowest ash and fat contents compared with those of PPC and AC samples. The pH values of CC and AC samples were similar and they were higher than those of PPC, while the acidity of CC was higher than those of PPC and AC samples which were equal. The microbiological testing showed that all the samples were safe and of high quality in terms of *Staphylococcus aureus, Escherichia coli,* and *Salmonella* determination, LAB was not detected in the AC sample, however, in the case of the CC sample presented a higher load in comparison with the PPC sample.

Keywords: cottage cheese, pasteurized processed cheese, analogue cheese, microbiological quality, physicochemical properties.

Résumé

Cette étude a étudié l'état physicochimique et microbiologique de trois types de fromages les plus consommés dans la ville de Guelma que sont le fromage blanc (CC), le fromage analogue (AC) et le fromage fondu pasteurisé (PPC), en déterminant la composition chimique (humidité, cendres, et graisses), les propriétés physicochimiques (pH et acidité) et la qualité microbiologique (Staphylococcus aureus, Escherichia coli, Salmonella et bactéries lactiques (LAB)). Les résultats ont montré que le CC avait la teneur en humidité la plus élevée et les teneurs en cendres et en graisses les plus faibles par rapport à celles des échantillons PPC et AC. Les valeurs de pH des échantillons CC et AC étaient similaires et supérieures à celles du PPC, tandis que l'acidité du CC était supérieure à celle des échantillons PPC et AC qui étaient égales. Les tests microbiologiques ont montré que tous les échantillons étaient sûrs et de haute qualité en termes de détermination de *Staphylococcus aureus*, *Escherichia coli* et *Salmonella*, (LAB) n'a pas été détecté dans l'échantillon AC, cependant, dans le cas de l'échantillon CC présentait une charge plus élevée en comparaison avec l'échantillon PPC.

Mots clés : fromage frais, fromage fondu pasteurisé, préparation fromagère, qualité microbiologique, propriétés physicochimiques.

الملخص

تناولت هذه الدراسة الحالة الفيزيائية والكيميائية والميكروبيولوجية لثلاثة أنواع من الجبن الأكثر استهلاكا في مدينة قالمة و هم الجبن الطازج، و التحضيرة الجبنية، و الجبن المبستر، وذلك من خلال تحديد التركيب الكيميائي (الرطوبة و الرماد و الدهون)، والخصائص الفيزيائية والكيميائية (الرقم الهيدروجيني و الحموضة)، والجودة الميكروبيولوجية (المكورات العنقودية الذهبية، الإشريكية القولونية، السالمونيلا، وبكتيريا حمض اللاكتيك). أظهرت النتائج أن الجبن الطازج يحتوي على أعلى محتوى من رطوبة والأقل محتوى من الرماد والدهون مقارنة بعينات الجبن المبستر و التحضيرة الجبنية. كانت قيم الرقم الهيدروجيني لعينات الجبن الطازج والتحضيرة الجبنية متشابهة وكانت أعلى من تلك الخاصة بالجبن المبستر، في حين كانت حموضة الجبن الطازج أعلى من تلك الموجودة في عينات الجبن المبستر والتحضيرة الجبنية التي كانت متساوية. أظهر الاختبار الميكروبيولوجي أن جميع العينات كانت آمنة وذات جودة عالية من حيث تحديد المكورات العنقودية الذهبية والإشريكية القولونية والسالمونيلا، ولم يتم اكتشاف بكتيريا حمض اللاكتيك في عينة التحضيرة الجبنية، ومع ذلك، في حالة عينة الجبن الطازج كان هناك حمولة أعلى بالمقارنة مع عينة الجبن المبستر.

الكلمات المفتاحية: الجبن الطازج، الجبن المبستر، التحضيرة الجبنية، الجودة الميكروبيولوجية، الخواص الفيزيائية والكيميائية.

List of abbreviations

Abbreviation	Meaning
AOAC	Association of Official Analytical Chemists
BC	Before Christ
BHIB	Brain Heart Infusion Broth
BSA	Bovine Serum Albumin
CFU	Colony-forming unit
e.g.	exempli gratia
EDTA	Ethylenediaminetetraacetic acid
EMB	Eosin Methylene Blue
EMB	Eosin Methylene Blue
g	Gram
g/mol	Grams per mole
h	Hour
i.e.	id est
JORA	Algerian Journal of the Official Republic
kDa	Kilodalton
L	Liter
LAB	Lactic Acid Bacteria
ml	Milliliter
mol/L	Molarity
MRS	De Man, Rogosa and Sharpe medium
N	Normality
NaOH	Sodium hydroxide

NMFG	native milk fat globule
NSLAB	non-starter lactic acid bacteria
nm	Nanometer
OD	Optical Density
rpm	Revolutions per minute
RV	Rappaport-Vassiliadis enrichment broth
spp.	species (plural)
SS	Salmonella Shigella agar
vs.	versus
w/w	weight by weight

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INTRODUCTION

Introduction

Cheese has a long history in the human diet as a source of critical nutrients, since it is a rich source of protein and nutritional elements (such as calcium and phosphorus), and is also necessary for the development of healthy bones and teeth, as well as providing essential fatty acids to the brain (Hammam *et al*., 2020; Dimitrova *et al*., 2020). Today, over 1,800 varieties grace our tables, each a unique expression of cultural heritage and production techniques, with regional variations reflecting the ingenuity of cheesemakers across the globe (Fox and McSweeney, 2004).

The types of cheese have quickly gained interest due to their pleasant organoleptic characteristics and thus constitute an interesting alternative to the consumption of milk, each cheese having its specification. They vary by the nature of the milk, by the fat content, and by their method of refining. Among the types of cheese that can be found: fresh cheeses (cottage cheese), pressed cheeses, hard cheeses, filata cheeses, soft cheeses, washed or bloomy rinds, as well as processed cheeses (Boutonnier, 2000).

The risk of possible spoilage of cheeses by different useful or pathogenic microorganisms requires rigorous microbiological and physicochemical monitoring from the milking of the cheese milk until cheese making (Ruqia *et al*., 2015). As a result of this context, our study is concerned with evaluating and monitoring the physicochemical and microbiological quality of the most consumed cheese in Guelma City.

The objective of this research is to compare the characteristics of cottage cheese, pasteurized processed cheese, and analogue cheese by analyzing their microbiological and physicochemical properties and understanding the contribution of the studied properties to the overall quality of cheeses.

CHAPTER 01

LITERATURE REVIEW

1. Definition of cheese:

Cheese is defined as a ripened or unripened product made by coagulation of the proteins in milk, skimmed or partly skimmed milk, cream, whey cream or buttermilk, or a combination of these liquid streams, and with a concentration of the proteins from the source material *(*Kindstedt, 2018). Raw or pasteurized bovine milk is used to make most cheese varieties, with buffalo, goat, and sheep milk using calf rennet as a traditional clotting agent containing chymosin enzyme (rennin) (Codex Alimentarius, 1973).

Cheese is considered as concentrated source of many of the nutrients in milk. During the usual cheese-making process, the water-insoluble components of milk (e.g. the milk protein - casein and fat) remain in the curd and the water-soluble constituents (e.g. carbohydrates, salts, and proteins smaller than casein) remain in the whey. The amount of various nutrients retained in the curd and whey depends on the type of cheese manufactured, the type of milk used, and the manner of coagulation. The enzymes and bacteria involved in the ripening of each specific kind of cheese may also alter the nutrient content of the end product (Enwa *et al.*, 2013).

1.1. Definition of Cottage Cheese

Cottage cheese is categorized as a cultured dairy product. Cottage cheese is the product obtained from coagulated milk, cream, partly or wholly skimmed milk, reconstituted (prepared) milk, or a combination of these products. Cottage cheese is a soft, un-ripened cheese containing about 80% moisture. Cottage cheese can also be made with various additions such as herbs, pickles, and spices (Catsber and Kempen, 1990).

1.2. Definition of Processed Cheese

In the broadest sense, this group of cheese products differ from natural cheeses in that they are not made directly from milk (or dehydrated milk), but rather from various ingredients. The two main categories, namely, pasteurized processed (also called process)

cheese products and analogue cheese, may be further subdivided depending on composition and the types and levels of ingredients used (Fox *et al*., 2017).

1.2.1. Pasteurized Processed Cheese

Guinee defined processed cheese as the cheese which can be produced by blending natural cheese of different ages and degrees of maturity in the presence of emulsifying salts and followed by heating and continuous mixing to form a homogeneous product (Guinee *et al*. 2004).

In pasteurized processed cheese, a minimum cheese content of ≥51 % (w/w) of the final product is required, in which non-cheese ingredients (e.g., dairy ingredients) can be used at a level up to ~15 % (Fox *et al*., 2017).

1.2.2. Analogue Cheese

Analogue cheese is made from mixtures of dairy and/or non-dairy proteins and fat/oils. Hence, it was suggested in response to increasing manufacturing costs of processed cheese, analogue cheese products have been developed to meet demand in fast food outlets, in formulated foods, and in school lunch programs (Tamime, 2011).

Analogue cheeses (ACs) are substitutes or imitations of natural cheeses or processed cheeses (McSweeney, 2007), these products are variously labeled as analogues, analogs, imitation, substitute, artificial, extruded, synthetic, Tofu and/or filled cheese (Tamime, 2011), that are manufactured by blending various edible oils/fats, proteins, other ingredients, and water into a smooth homogeneous blend with the aid of heat, mechanical shear, and emulsifying salts (McSweeney, 2007).

2. History of Cheese:

Cheese is one of the most ancient forms of manufactured food. It is thought that cheese-making could go as far back as 10,000 BC when sheep and goats were first domesticated in the Middle East and early herdsmen would have consumed milk. Due to contaminating

bacteria, milk has a short shelf life, especially in warm climates (Walther *et al.,* 2008); therefore, sour milk naturally separates into curds and whey, the solid curd providing an edible and nourishing food. It is likely that nomadic tribes, spread from the Middle East to Western Europe and South and Central Asia, found animal-skin bags a useful way to carry milk on animals' backs when on the move.

Cheese produced in Europe, where climates are cooler than in the Middle East, requires less salting for preservation. With less salt and lower acidity, cheese was a suitable environment for various beneficial microbes and molds, giving aged cheeses their pronounced and interesting flavors (Walther *et al*., 2008).

- **Cottage cheese**: It is thought to be the first cheese made in America. For centuries, the European farmers made fresh farmhouse cheeses with naturally soured milk, after separating the curds from the whey. Immigrants to America brought the tradition of fresh cheese-making with them and by the mid-1800s the term cottage cheese entered the American vocabulary. Cottage cheese is sold both plain and with added flavorings such as fruit and herbs (Kansas Foundation for Agriculture in the Classroom, 2024).

- **Processed cheese:** A dairy item often made from natural cheeses, emulsifying salts, and, inevitably, other dairy or non-dairy components, it has a history dating back to the early part of the 20th century. Looking for new procedures for a modern make of cheese with a longer shelf-life, bypassing conventional strategies such as air-drying or smoking, Jan Hendrikzoon invented a heat-treatment method for canned Gouda cheese in 1899. The Swiss researchers Walter Gerber and Fritz Stettler in 1911 (El Dakhakhny and Dabour, 2016) found that pasteurization led to insufficient results, so they followed traditional fondue preparation (Mulsow *et al*., 2007). The idea possibly originated from the Swiss national dish, Fondue, for which cheese is heat-treated (melted) in the

presence of wine, which contains tartrate that has an emulsifying effect (Carić and Kaláb, 1999), to get a stable but modified product. It was noticed also, that other salts may be used and, due to their function, these were referred to as 'melting salts' or 'emulsifiers' (Mulsow *et al*., 2007).

3. Classification of Cheese:

Cheese is one of the most complex and diverse foods enjoyed today. Certainly, the characteristics and activity of the specific starters and adjunct cultures selected for each variety contribute to the complexity and diversity of cheeses. In addition to the microbiological aspects, features contributing to the diversity and differentiation of cheese include the variability among fundamental processing and aging characteristics that influence both the chemical composition of the fresh cheese and its enzymatic potential during ripening (Donnelly, 2014).

Multiple cheese classification models exist, and broad studies have previously analyzed the majority of the schemes developed to classify cheese varieties into meaningful groups of families (Fox *et al*., 2000; McSweeney and Ottogalli, 2004).

The classification proposed by Lenoir *et al.* (1985) shows in Figure 1 how the diversity of French cheeses is mainly due to differences in three key processing steps (coagulation, draining, and ripening) that define the type of technology and major chemical characteristics of each cheese variety. These three key processing steps define the basis for the diversity and differentiation of cheeses but also indirectly influence each other.

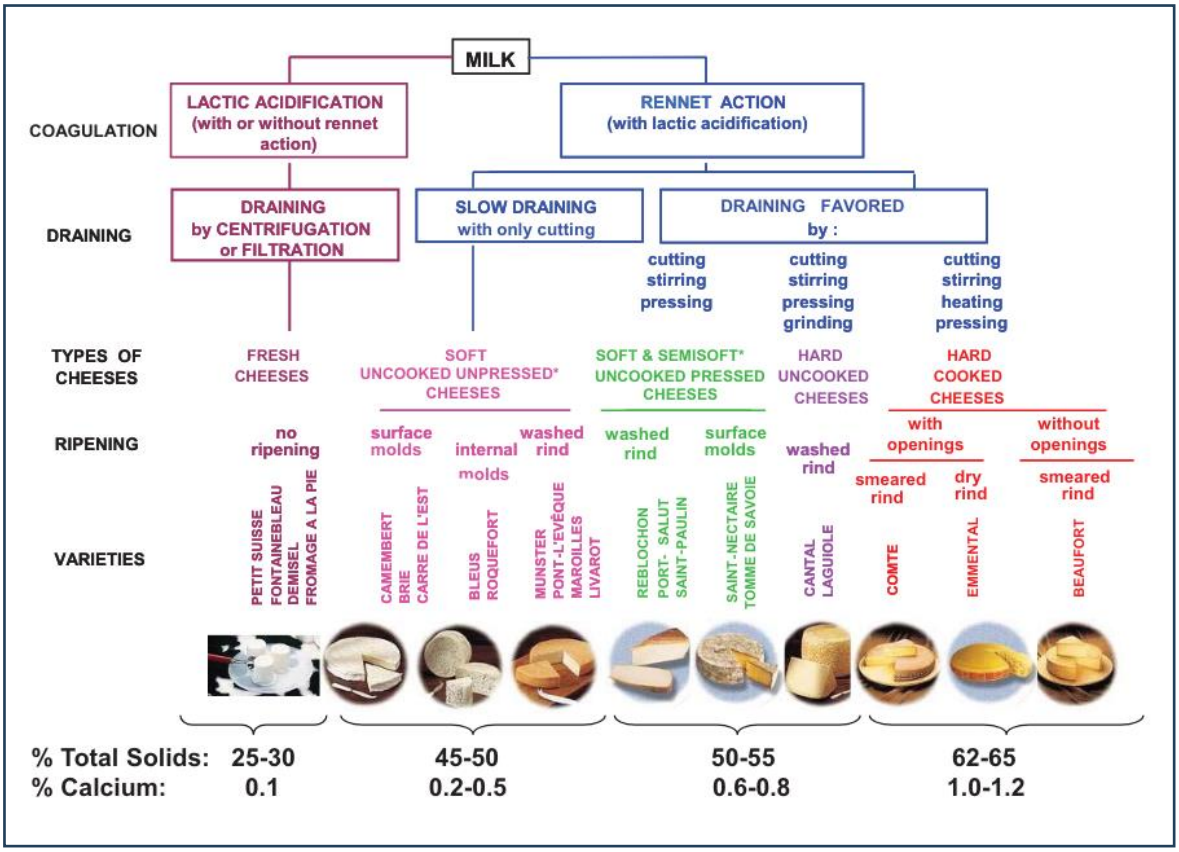


Figure 1: Cheese Classification based on processing (Lenoir *et al.*, 1985)

Almena *et al.* (2001) created a modified version of the original model of Lenoir *et al.* (1985) presented in Figure 1. This revised version of the original model represents a more comprehensive approach because it also integrates the nature of raw ingredients and the characteristics of the coagulation and main processing steps, as well as a more specific description of the textural properties of the cheese.

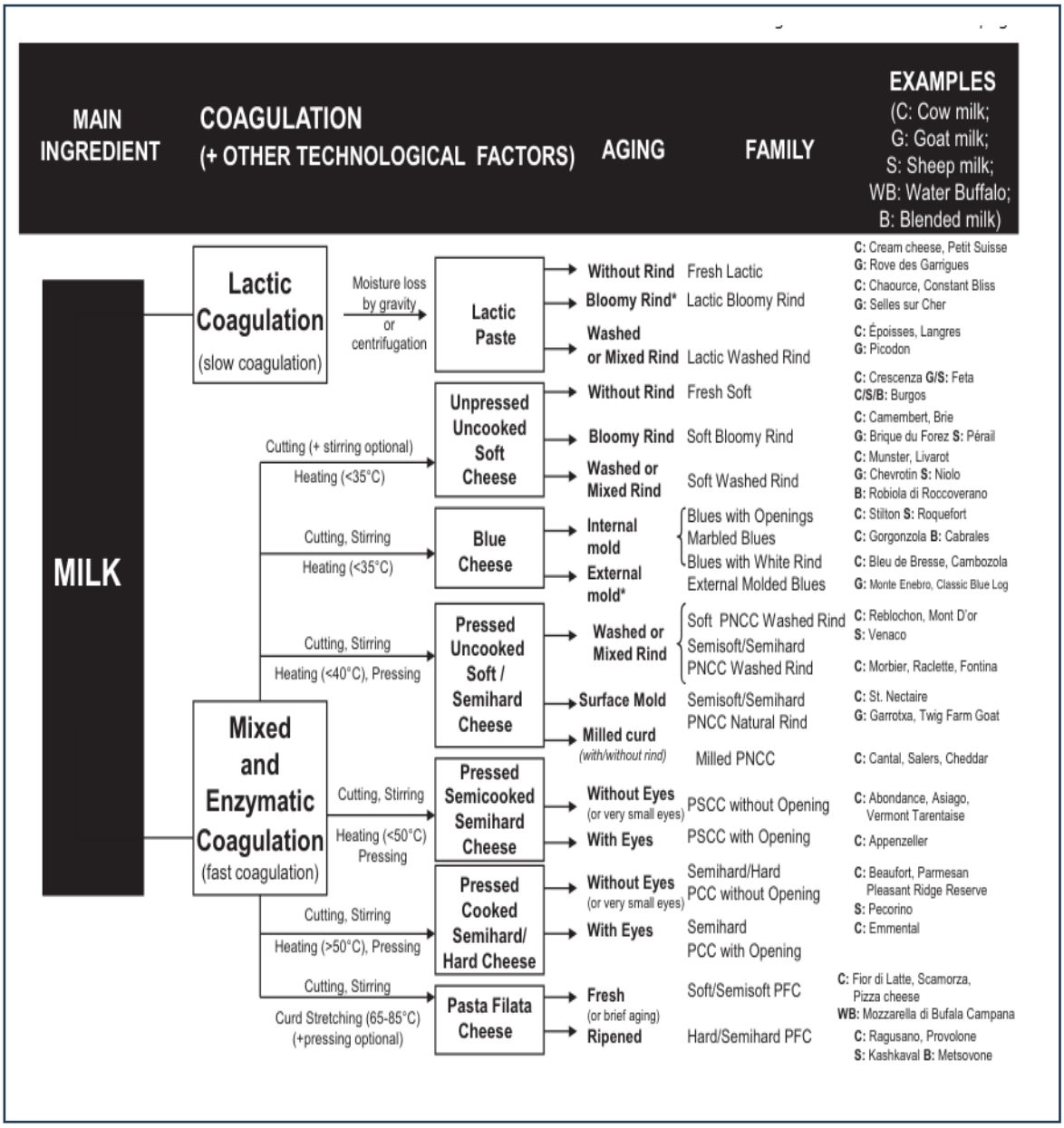


Figure 2: Classification of Cheese Based on Raw Ingredient (Almena-Aliste and Mietton, 2014)

4. Cheese Structure

Caseins, the main structural component of cheese, are present in the form of a network in the cheese matrix in which fat globules, water, minerals, bacteria, and dissolved solutes such as lactose, lactic acid, soluble salts, and peptides are all interspersed. The spatial

arrangements of these components and their interactions determine the structure of the cheese (Guinee, 2016).

4.2. Structure of Cottage Cheese:

Cottage cheese is manufactured from skimmed milk by coagulation with lactic acid bacteria and a small amount of a coagulant to the isoelectric pH (4.6) of casein. This produces a skimmed curd that is cooked at 50°C, cooled, and dressed with salted cream.

The cooking process would intuitively suggest that the curd has a denser protein matrix, but not so thick as to prevent whey drainage from within the curd particle. The protein matrix is similar to yogurt but denser. Filaments from lactic acid bacteria have been observed, attached to the protein matrix. Fat globules of cottage cheese are embedded into the protein matrix (Everett, 2007).

4.3. Structure of Processed Cheese:

Processed cheese is a complex system consisting of protein, fat, salts, water, and other ingredients. Various properties of processed cheese products are affected by the composition and nature of the raw natural cheese, the nature and amount of the emulsifying salts, and the manufacturing process (Mulsow *et al.,* 2007).

According to Heertje *et al*. (1981), the microstructure develops in two different stages: the casein matrix of the raw material is disaggregated into subunits, and the gelation stage is responsible for the formation of string-like structures consisting of dissociated protein fragments.

The spherical fat droplets are embedded in a continuous protein matrix. The fat and para-caseinate in processed cheese are distributed more homogeneously than in natural

cheese. The mean fat globule size varies greatly, depending on the type and concentration of the emulsifying salts used as well as on processing conditions (Mulsow *et al*., 2007).

4.4. Role of Structural Elements on Functional Properties of Cheese:

4.4.1. Protein Phase:

The formation of a protein network is a crucial step in cheese manufacture. The destabilization of casein micelles is one of the first steps in the manufacture of cheese (Dalgleish and Corredig, 2012). The destabilized casein micelles aggregate into chains and clusters, leading to the formation of a 3-dimensional gel. Several studies have reported that the factors, such as concentration of casein (Karlsson *et al*., 2007), properties of casein micelle like its size (Logan *et al*., 2014), and coagulation conditions (pH, temperature, and rennet concentration) (Wium *et al*., 2003; Ong *et al*., 2011a, 2012), can all influence the coagulation process. This may influence the arrangement of casein into the protein matrix and also the microstructure and the quality of the final cheese (Ong *et al*., 2012).

In other studies, the coarseness of the protein network of the gel or cheese increased with increasing coagulation temperature (Wium *et al.,* 2003; Ong *et al.,* 2011a).

4.4.2. Fat Phase:

During cheese manufacture, milk fat globules are entrapped within the protein gel network, and processes such as scalding, cheddaring, hot water stretching, and pressing, can cause aggregation, coalescence, and disruption of the fat globules. As shown in Figure 3, in the cheese matrix, fat globules can exist as intact (spherical fat globules covered with native membrane materials), aggregated (clumps of circular fat globules), coalesced (spherical but larger than typical milk fat globules), elongated (especially in pasta-filata cheese-types), or even non-globular forms (Michalski *et al.,* 2007; Rogers *et al.*, 2010; Ong *et al.,* 2011b).

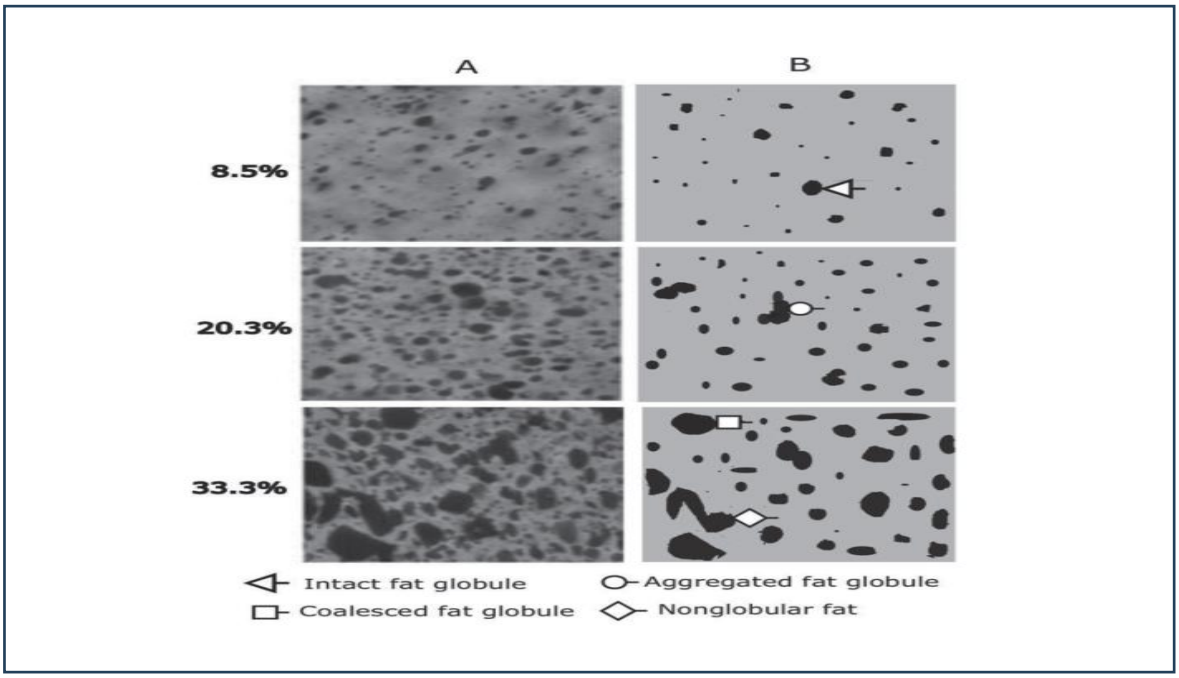


Figure 3: Column A Shows the Confocal Laser Scanning Microscopy Images (100 µm × 100 µm) of Cheddar at 3 Different Fat Levels. Column B is the Schematic Representation of the Cheddar Cheese Microstructure of Fat Globules (Rogers *et al.,* 2010)

The microstructure of fat globules can influence the physical properties of cheese. Several factors, such as fatty acid compositions, native milk fat globule (NMFG) size, level of fat, and properties of fat globule membrane materials, can influence various properties of cheese. The fatty acid composition of milk fat can alter the rheological and textural properties of cheese (Mansson, 2008). Palmitic acid (C16:0) and oleic acid (C18:1) are the major saturated and unsaturated fatty acids in milk that have high and low melting points, respectively *(*Coppa *et al.,* 2011*)*; a higher ratio of C18:1 to C16:0 is known to produce more creamy and less firm cheese (Coppa *et al.,* 2011; Bocquel *et al.,* 2016).

4.4.3. Aqueous/Serum Phase:

Water in cheese can be broadly classified as bound or bulk water. Bound water is strongly associated with protein and other components of the cheese matrix, and this water is not available as a solvent, whereas bulk water is loosely associated with the protein matrix,

retains a large solvent capacity, and is freezable at −40°C (McMahon *et al.,* 1999). Bulk water may be either present within the channels surrounding the fat (free water) or entrapped within the protein matrix (entrapped water). The distribution and state of water in cheese depend on factors such as cheese type and age (Kuo *et al.,* 2001; Smith *et al.,* 2017). Cheese generally becomes softer as the levels of moisture increase (McMahon *et al.,* 2005).

4.4.4. Gas Phase:

Cheese matrix structure, the rate and extent of gas production and its behavior in the cheese matrix are known to play an important role in desirable eye formation (Daly *et al.,* 2010). Carbon dioxide in the cheese matrix is mainly produced due to lactate fermentation by propionic acid bacteria during the warm room ripening (Daly *et al.,* 2010).

5. Cheese Making Technology

5.1. Cottage Cheese

Cottage cheese is categorized as a cultured dairy product. It is obtained from coagulated milk, cream, partly or wholly skimmed milk, reconstituted (prepared) milk, or a combination of these products. Cottage cheese is a soft, unripened cheese containing about 80% moisture. Cottage cheese is classified as follows (Catsber and Kempen, 1990):

- Full-fat cottage cheese: it has a fat in dry matter content of at least 45% but not more than 60%.
- Medium-fat cottage cheese: it has a fat in dry matter content of at least 25 % but not more than 45 %
- Low-fat cottage cheese: it has a fat in dry matter content of at least 10 % but not more than 25 %.
- Fat-free cottage cheese may also be named skim milk cottage cheese, which has a fat in dry matter content of at least 10 %.

5.1.1. Milk Preparation for the Manufacturing of Cheese

5.1.1.1. Separation and Standardization of Milk for Cheese

The cream fraction of raw milk is separated from the skim milk by passing pre-heated raw milk (45 – 60°C) through a conventional or hermetic centrifugal separator. Standardization follows directly after separation and involves the adjustment of the fat content of milk to obtain a product with a defined fat content. Standardization is preceded by separating the milk and cream and then remixing the two fractions in the desired proportions (Horwood, 1995).

5.1.2. Pasteurization of Milk for Cottage Cheese

Pasteurization is a mild heat treatment that destroys all vegetative pathogens and heat-sensitive enzymes. The milk is heated to 72°C, followed by rapid cooling. Milk pasteurization's purpose is to eliminate microbial competition for the starter culture (Tamine and Robinson, 1985).

5.1.3. Curd Formation

A bacterial culture is added to the milk to acidify it. The temperature (29°C) creates ideal conditions for milk coagulation and for the bacteria to grow, and the acidic environment prevents foreign bacterial contamination. Rennet is added to the milk when it reaches a certain pH to precipitate casein protein (Tamine and Robinson, 1985).

The coagulum is cut with vertical and horizontal knives into small cubes. This eases the whey separation (syneresis) by increasing the exposed surface areas. The cubes of curds and whey are gently stirred to prevent lump formation (Tamine and Robinson, 1985).

5.1.4. Scalding of Cottage Cheese Curds

Scalding is a heat treatment given to cheese curds to regulate the texture and acidification of the curd. The curds are slowly heated to 55 °C to accelerate the whey separation. Slow heating is necessary to prevent case hardening. The curd-whey mixture is stirred as soon as the required temperature is reached to ease the separation.

5.1.5. Draining of Whey from Cottage Cheese

Draining removes excess free moisture from a product by gravitation force to obtain partial drying of the product surface. The whey and the curd have separated due to cutting, stirring, and scalding. The whey is drained off either through a tap or by gravity separation (Tamine and Robinson, 1985).

5.1.6. Washing Curds in Cheese-making

Washing curds is a technique used in the production of certain cheeses, particularly washed-rind cheeses. Here's a breakdown of the process:

- **Washing:** The curds are washed 2-3 times with potable water at progressively cooler temperatures: 30-32°C, 14°C, and 4°C (Tewes, 2023). This cools the curds, removes some of the lactose (milk sugar), and reduces the acidity (Tewes, 2023). Less lactose means less food for the bacteria, which in turn inhibits further acid production during fermentation (Tewes, 2023).
- **Draining:** After washing, all the wash water is drained from the curds (Tewes, 2023).

5.1.7. Seasoning (Optional)

Salt and other optional seasonings like peppercorns and herbs may be added at this stage (Tewes, 2023).

5.1.8. Filling and Sealing

- In large-scale production facilities, the containers are filled with cheese using automated injection systems (Tewes, 2023).
- Smaller cheesemakers may fill and seal the containers by hand (Tewes, 2023).
- Suitable containers include plastic tubs with lids or foil seals (Tewes, 2023).
- Regardless of the filling method, the containers must be hermetically sealed to prevent spoilage and then stored at 4°C (refrigerator temperature) (Tewes, 2023).

5.1.9. Labelling

All the cottage cheese containers are pre-labeled or labeled after they are filled and sealed. (Tamine and Robinson, 1985).

5.2. Pasteurized Processed Cheese

The term "processed cheese" commonly describes a dairy product that is produced by heating a mixture of various cheese types with different degrees of ripening in the presence of appropriate emulsifying salts (mostly sodium phosphate, polyphosphates, citrates and/or their combinations) (Oliveira *et al*., 2016). The main processing steps involved in processed cheese manufacture are illustrated in Figure 04. The processing steps can be divided into two main stages:

a. **Ingredient selection and formulation:** The choice of the types of cheeses and their degree of ripening for the production of processed cheeses is substantiated by the sensory and physical properties expected (Oliveira et al., 2016). Fat standardization, and selection of appropriate emulsifying salts and other ingredients (cream and/or butter oil), to meet the targeted gross chemical composition (El Dakhakhny and Dabour, 2016).
b. **Processing and storage:** The production of processed cheese is usually performed under reduced pressure (vacuum) with constant stirring and at temperatures ranging from 72°C

to 145°C until a smooth and homogenous compact mass of curd is formed with the desired textural properties (Oliveira et al., 2016) then, packaging, cooling, cartooning, and storage (El Dakhakhny and Dabour, 2016).

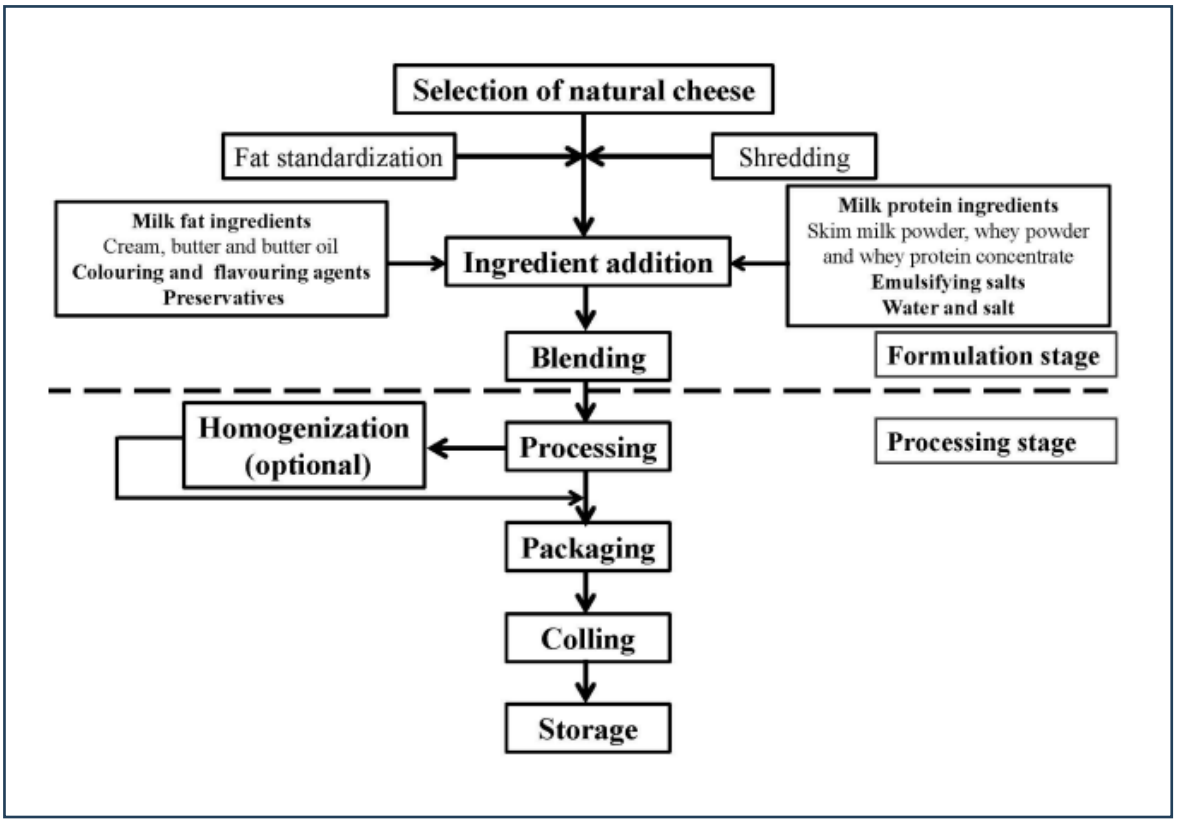


Figure 4: Schematic Flow Chart Showing Pasteurized Processed Cheese Manufacturing Steps (El Dakhakhny and Dabour, 2016)

5.3. Analogue Cheese

The manufacture of analogue cheese is similar to pasteurized processed cheese and involves formulation, processing, and packaging. A typical formulation (Table 1) shows that it differs from pasteurized processed cheese in that cheese is not normally included (Fox *et al*., 2017).

Table 1: Typical formulation for analogue cheese (Fox *et al*., 2017)

Ingredient	Level added (g/100 g blend)
Casein/caseinates	23.00
Vegetable oil	25.00
Starch	2.00
Emulsifying salts	2.00
Flavour	2.00
Acid regulator	0.4
Colour	0.04
Preservative	0.10
Water	38.50
Condensate	7.00

However, some cheese may be introduced as a flavoring agent or as required by customer specifications for label declaration. While production methods vary somewhat, a typical manufacturing procedure (Figure 5) involves the following steps (Fox *et al*., 2017):

a. **Formulation:** Selection of ingredient types and quantities to give the desired end-product characteristics (composition, flavor, texture, and cooking properties)
b. **Blending of ingredients:** The procedures differ concerning the sequence of ingredient addition. In some cases dry ingredients (e.g., casein, emulsifying salts) are added followed by oil and finally water, while being constantly agitated; but in others, oil and water are first added, followed by the dry ingredients.
c. **Processing the blend**
d. **Hot packaging**

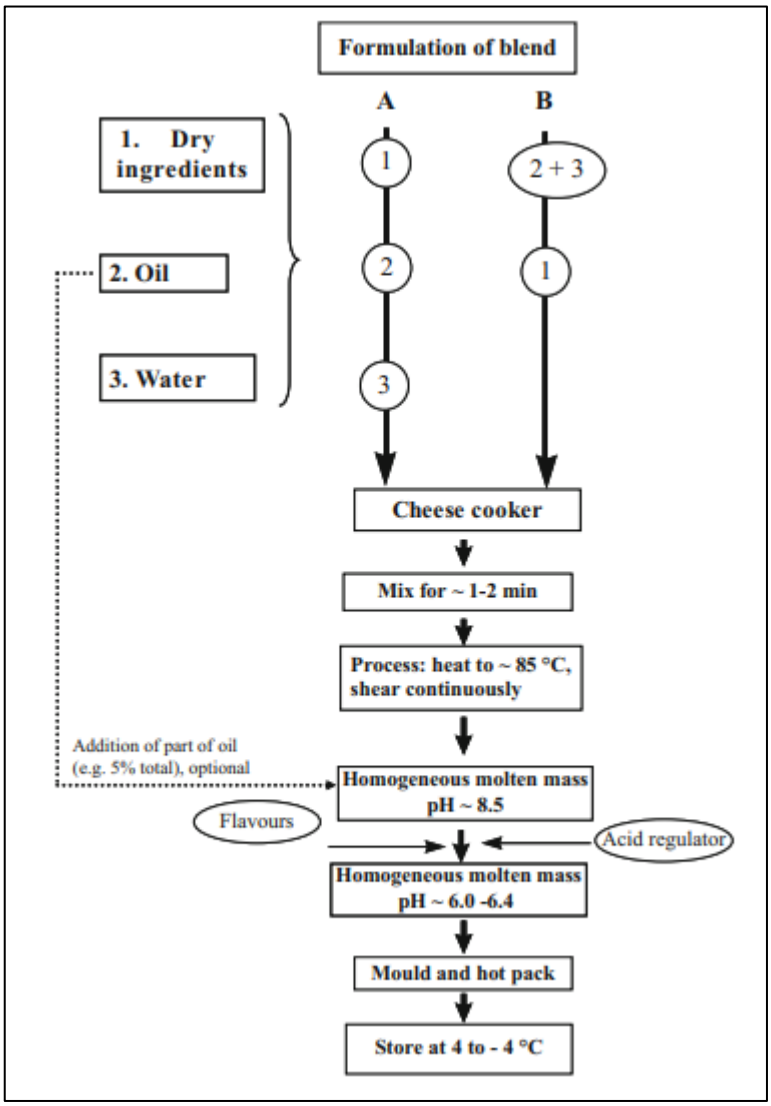


Figure 5: Typical manufacturing procedures (A, B) for analogue cheese (Fox *et al.*, 2017)

6. Bacterial Quality of Cheese

Cheese is essentially a microbial fermentation of milk. The microbial flora of the cheeses is significantly influenced by manufacturing and ripening methods, type of milk, water activity, salting methods, and gross chemical composition of the resultant cheeses (Hayaloglu, 2015).

6.1. Starter Bacteria

In most cheeses, selected strains of LAB, are previously grown in milk or another medium and added deliberately to the milk at the beginning of cheese manufacture. They are called 'starters' because they start lactic acid production and are divided into two types (mesophilic and thermophilic). The cultures are generally selected for their ability to produce lactic acid at a rate appropriate for the cheese being made, to resist attack by a bacteriophage, and to produce a cheese with the desirable flavor (Hayaloglu, 2015).

The initial number of starter bacteria in cheese milk, depending on the cheese type and they rapidly reach numbers of 109 CFU/g in almost all cheeses within a few hours of being added to the milk. Therefore, starter bacteria are the dominant organisms in cheese at the beginning of ripening (Hayaloglu, 2015).

6.2. Non-starter Lactic Acid Bacteria

All cheeses ripened for any length of time, except unripened cheeses, contain non-starter lactic acid bacteria (NSLAB). They mainly comprise facultatively hetero-fermentative lactobacilli, and obligately hetero-fermentative Lactobacillus spp, are occasionally found. Raw milk and/or the factory environment are the major sources of NSLAB in cheese. Small numbers of lactobacilli survive pasteurization and the high cooking temperature (52 C) is used in making some hard cheeses. Most NSLAB are salt-and acid-tolerant, facultative anaerobes and therefore grow quite well in cheese (Hayaloglu, 2015).

6.3. *Staphylococcus aureus*

Staphylococcus aureus belongs to the family Micrococcaceae, characterized as facultative aero-anaerobic, catalase-positive, non-spore-forming, gram-positive, coagulase-positive (Martínez-Vasallo *et al*., 2019). Staphylococcal food poisoning is one of the most common foodborne diseases worldwide (Jamali et al., 2015) and cheese contamination sources by *Staphylococcus aureus* are related to the cheese-making environment, including

equipment, personnel, water, coagulant agents or contamination between final products and raw materials (Jørgensen *et al*., 2005).

Cross-contamination of raw milk cheeses with S. aureus can occur through inadequate food handling practices. Unpackaged cheeses are particularly vulnerable to cross-contamination from other foods and utensils. On the other hand, the ability of this bacterium to survive and/or grow in cheese is largely dependent on the manufacturing steps during cheese making, the physic-chemical characteristics of the cheese (pH, salt content, water activity), and the growth requirements of the microorganisms (Kousta *et al*., 2010).

6.4.Escherichia coli

Escherichia coli (E. coli) are well-known gram-negative bacteria of the normal gastrointestinal flora of a wide range of warm-blooded animals. Although non-pathogenic, some strains can exhibit virulence factors leading to human illness, widespread pathogens associated with severe foodborne disease (EFSA, 2020).

Generic E. coli testing is used in the dairy industry to indicate unsanitary conditions in which the product was manufactured, including post-processing contamination and, much less likely, the possibility of pasteurization failure, because gram-negative bacteria in general, are inactivated by pasteurization (Trmčić *et al*., 2016). Although environmental and direct person-to-person or animal-to-person infections are also confirmed, through fecal matter during milking (Condoleo *et al*., 2022).

6.5. Salmonella

Salmonella belongs to the family of Enterobacteriaceae. They are Gram-negative and facultatively anaerobic. *Salmonella* is pathogenic for humans, causing abdominal pain, diarrhea, nausea, vomiting, chills, and fever are common symptoms; dehydration, headache, and prostration also may occur (El-Gazzar and Marth, 1992).

Salmonella spp. are the main target foodborne pathogens evaluated in studies performed with cheeses, *Salmonella* is one of the four key global causes of diarrheal diseases and it causes annually 93.8 million cases of foodborne illness and 155,000 deaths. Many salmonellosis outbreaks linked to the consumption of cheeses have been reported worldwide, *Salmonella* can enter the milk and dairy environment from many sources. Nevertheless, adequate milk pasteurization and hurdles inactivate this pathogen (Lobacz and Zulewska, 2021)

7. Cheese and Health Aspects

- **Protective effect for dental caries:**

Cheese decreased the dental caries coefficient. The studies showed an inhibitory effect of cheese on the metabolism or survival of cariogenic bacteria such as Streptococcus mutans. Casein also plays an important role, micellar casein selectively modifies the microbial composition of dental plaque. Casein phosphopeptides react with high concentrations of calcium and phosphate to form calcium phosphate complexes. These complexes lead to remineralization of the enamel and are incorporated in toothpaste, gels, and chewing gum (Walther *et al*., 2008).

- **Weight reduction or anti-obese effects:**

The recommended nutritional interventions for weight reduction vary among low-fat, low-carbohydrate, and other models. When the low-fat approach is argued, milk, dairy products, and especially (full-fat) cheese are usually condemned because of their high-fat content. Therefore, dairy products are often omitted by people trying to lose weight. Furthermore, milk and cheese are high in protein, and helpful in a calorie-restricted diet (Walther *et al*., 2008).

- **Anti-hypertensive property:**

In several studies, dairy products have shown a beneficial effect on blood pressure, especially in mildly hypertensive subjects. Two main components seem to be relevant in this situation: calcium and bioactive peptides. A diet with (low-fat) dairy products seems to reduce the incidence of hypertension with a significant effect on the primary prevention of cardiovascular diseases. Besides calcium, potassium, and magnesium also seem to have a beneficial effect on blood pressure. This is another reason why dairy products, as a good source of all three minerals, are recommended to reach the recommended daily allowance of calcium (Walther *et al*., 2008).

CHAPTER 02

MATERIAL AND METHODS

2.1. Questionnaire:

Before starting our work, a questionnaire based on the most consumed cheese among people in Guelma City was used (Appendix 1).

The analysis of the data collected makes it possible to draw up the following results (Figure 6):

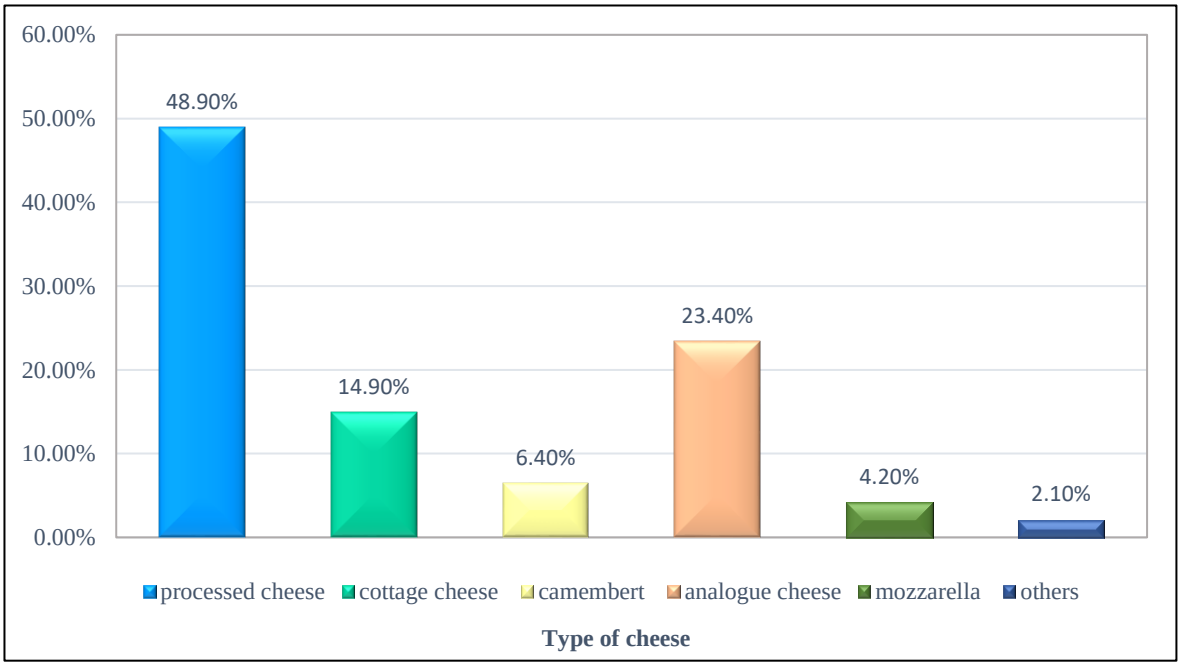


Figure 6: The Distribution of Consumption Preferences by Type of Cheese

Following the questionnaire on cheese consumed among people in Guelma City, including options such as processed cheese, mozzarella, cottage cheese, cheddar, and camembert, as well as other types like Gouda cheese, it determined participants' cheese preferences. The results revealed that processed cheese was the most consumed, with a percentage of 48.9%, making it one of the most popular choices among respondents. Then, analogue cheese was in second place with 23.4%, followed by cottage cheese with 14.9%, camembert, mozzarella, and other types obtained the lowest percentages of 6.4%, 4.2%, and

2.1% respectively. Thus, processed, analogue, and cottage cheese were chosen as study samples.

2.2. Collection and Preparation of Samples

The cheese samples were purchased from a market in Guelma City, for each cheese type a brand was chosen randomly. Five boxes from each type were purchased, provided that all the boxes for each type of cheese were from the same batch, therefore, a total of 15 samples were the subject of the study.

All samples were purchased at 11:00 a.m., they were transported in a cooler to the laboratory. The samples were stored in the freezer until the time of analysis (microbiological analysis was performed as soon as possible).

Analytical results for each cheese were obtained, presented, and interpreted separately and compared to results obtained by similar or related scientific researchers.

2.3. Proximate Analysis

Two replicates were made for each sample for the following tests: Moisture, fat, ash, protein, and carbohydrate

2.3.1. Moisture Content and Dry Matter

According to AOAC (2000), the oven drying method determined moisture content, where 10 grams of all the samples were heated in an air oven for 6 hours at 102°C. Moisture percent was calculated using the following equation:

$$\text{Moisture\%} = (\text{Loss in weight}/\text{Sample weight}) \times 100$$

2.3.2. Fat Extraction

Crude fats of cottage cheese, processed cheese, and analog cheese were obtained according to the AOAC (2000) Soxhlet method. Soxhlet glassware was fitted to a weighed distillation flask containing diethyl ether solvent and placed in the heater of the apparatus, the solvent was continuously volatilized and then condensed into the condenser apparatus to pass through the 10 g moisture-free ground sample placed into the filter paper and then in the extraction thimble.

After extraction, heating was stopped, the solvent was collected in the condenser apparatus and removed, and the crude fat left in the distillation flask was allowed to cool down and weighed. Fat% was calculated using the following equation:

$$\text{Fat \%} = (\text{weight of the fat in the sample/weight of dried sample}) \times 100$$

2.3.3. Determination of Ash

The sample's Ash content was determined using the dry ashing method (AOAC, 2012). Five grams of the food sample was put in a weighed crucible and placed in a muffle furnace (Carbolite CWF 1100) at 600°C for 5 to 6 hours until they became completely free from carbon (i.e., a light gray or white color ash). Then, the crucible (with its content) was cooled in a desiccator at room temperature and weighed. Ash contents were calculated as follows:

$$\text{Ash \%} = (\text{Weight gain by the dish /Weight of the sample}) \times 100$$

2.4. Physico-chemical Analysis

2.4.1. pH Determination

The pH of the samples was determined according to Al Assoly et al. (2019) by blending 10 gm of each sample with 100 ml of distilled water (1:10), and homogenizing for 2 minutes, then measuring the pH of the homogenate in triplicate runs using a pH meter.

2.4.2. Titratable Acidity Determination

The acidity of cheeses is determined using the method (AOAC 920.124). Ten grams of each cheese are placed in a beaker containing 50 ml of distilled water previously heated at 40°C. The mixture was centrifuged at 6000 rpm for 10 minutes. After centrifugation, the supernatant is transferred into a volumetric flask, and the volume is adjusted to 105 ml with distilled water. Two drops of phenolphthalein were added to 25 ml of the diluted supernatant, and then titrated with NaOH (0.1 N), the volume of NaOH used was noted.

The acidity is calculated using the following formula:

$$\text{Titratable acidity \% (as lactic acid)} = [V\ (NaOH) \times N \times 90.05] / m\ (g)$$

Where:

- m (g): sample weight;
- V (NaOH): Volume in milliliters of NaOH solution;
- N: Normality of NaOH solution (mol/l);
- 90.05: Molar mass of lactic acid (g/mol).

2.5. Microbiological analysis

This part aimed to conduct microbiological analysis by looking for different germs, which were then compared with the standards established by the official journal. According to the official journal (2017), the germs were:

- *Escherichia coli*
- Coagulase-positive *Staphylococcus*
- *Salmonella*

Also, a microbial analysis for lactic acid bacteria (LAB) was carried out for all the samples.

All materials used for microbiological analysis were previously sterilized by autoclaving at 121°C for half an hour.

2.5.1. Preparation of Mother Solutions and Dilutions

Ten grams of each sample were weighed aseptically and homogenized in 90 ml of diluent (peptone water) to have a mother solution. The mother solution was left for 30 minutes to ensure the germs were revived. Aseptically, 1 ml of the 10^{-1} dilution (mother solution) was transferred into a test tube containing 9 ml of sterile diluent (peptone water), this made 10^{-2} dilution. All other decimal dilutions were thus prepared until the required dilution was obtained (Figure 7). Each dilution test tube was vortexed before conducting the next one (Harrigan, 1998). The dilutions were made for E. coli, coagulase-positive staphylococcus, and LAB.

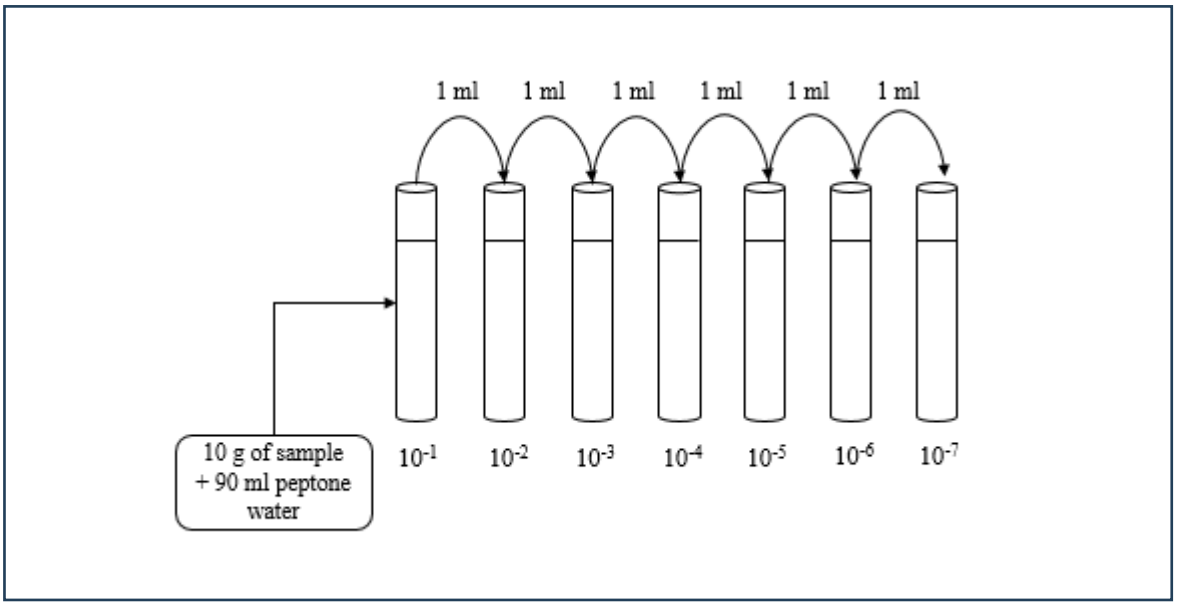


Figure 7: Preparation of Decimal Dilution

2.5.2. Enumeration of E.*coli*

- **Purpose**

E.*coli* is found in the small intestine of most warm-blood animals, its presence in milk is therefore an indication of fecal contamination. E.coli is responsible for spoiling cheese due to its ability to ferment lactose rapidly to produce lactic acid and acetic acid (Walstra *et al*., 1993).

- **Principe**

Based on detecting the bacteria in a media sample using standardized microbiological techniques. The plate count technique is the most common method for E. coli detection and enumeration (Figure 8). This method involves inoculating the sample on a specific culture medium for E. coli, Eosin Methylene Blue (EMB), and setting up serial dilutions. After incubation, the colonies of E. *coli* are counted to determine the number of colonies formed on the plate and estimate the concentration of the bacteria in the initial sample (Hachimi and Duoudi, 2019).

- **Procedure**

- 15 ml of EMB medium was poured into sterile Petri dishes and allowed to solidify.

- 0.1 ml of each dilution (10^{-1}, 10^{-2}, and 10^{-3}) was taken and introduced aseptically into single-use Petri dishes.

- The sample dilution was homogenized and spread by circular movements, then incubated at 37°C for 48h.

- Characteristic colonies appeared bright metallic green (Murray et al., 2017).

- **Reading results**

-Dishes containing 30 to 300 colonies were retained.

-The number of microorganisms was calculated using the following formula:

$$N = \frac{\Sigma \text{ colonies}}{v \times (n1 + 0.1\, n2) \times d1}$$

Where:

V: the inoculum volume applied to each dish (generally expressed in ml).

n1: Number of dishes used for the first dilution;

n2: Number of dishes used for the second dilution;

d: Dilution factor from which the first counts were obtained

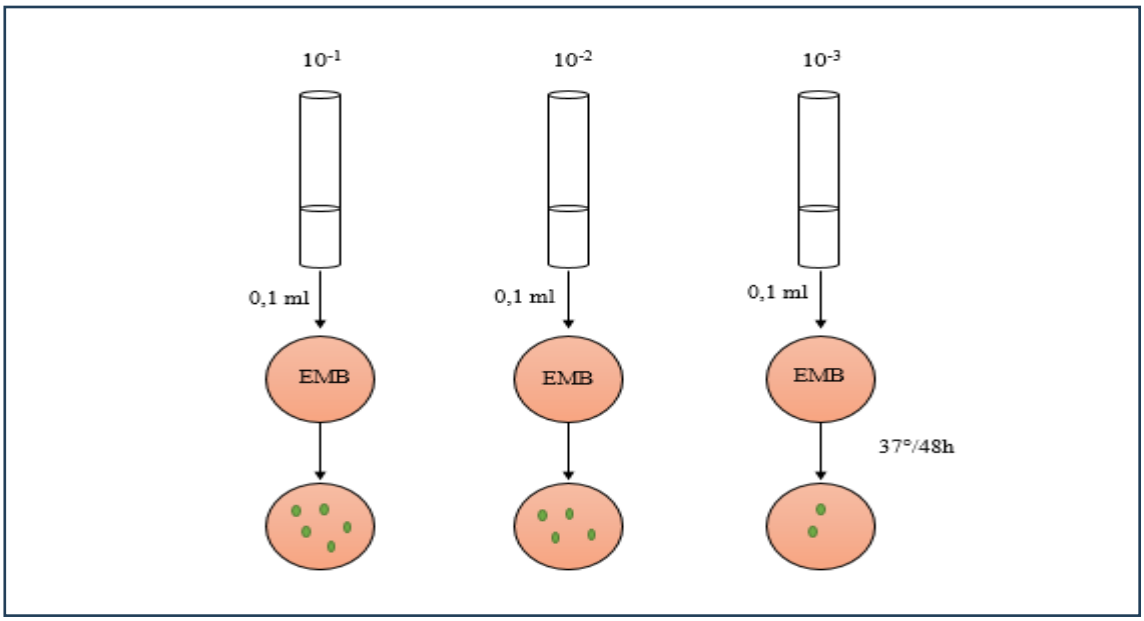


Figure 8: Enumeration of E.*coli*

2.5.3. Enumeration of Coagulase-positive *Staphylococcus*

- **Purpose**

Staphylococcus aureus is a common cause of food-borne intoxication (Johler *et al*., 2018). The occurrence of S. aureus in the cheese production chain since milk is subjected to low-temperature processing without the pasteurization process and enters the food chain during the processing and preparation of the products (Gajewska *et al*., 2022).

- **Principe**

It consists of highlighting and quantifying the presence of this bacteria in cheese samples (Smith *et al*., 2018).

- **Procedure**

It was done on Chapman agar from the decimal dilutions tubes (10^{-1}, 10^{-2}, and 10^{-3}) and incubated at 37°C for 24 hours.

- **Reading results**

Consider representative Staphylococcus aureus colonies that appear golden in color and small in size (Figure 9). To confirm the identification of Staphylococcus aureus, it is recommended to perform the coagulase test (Mekhloufi, 2018):

-A well-isolated Staphylococcus aureus colony was inoculated into a sterile tube containing Brain Heart Infusion Broth (BHIB) and then incubated at 37°C for 24 hours.

-After incubation, aseptically an equivalent volume of the broth and plasma (v/v) was transferred in a sterile tube, and the mixture was incubated at 37°C for 16 to 24 hours.

- All the mixtures that have formed a coagulum are considered a positive coagulase test, and the bacteria was staphylococcus aureus (JORA, 2017).

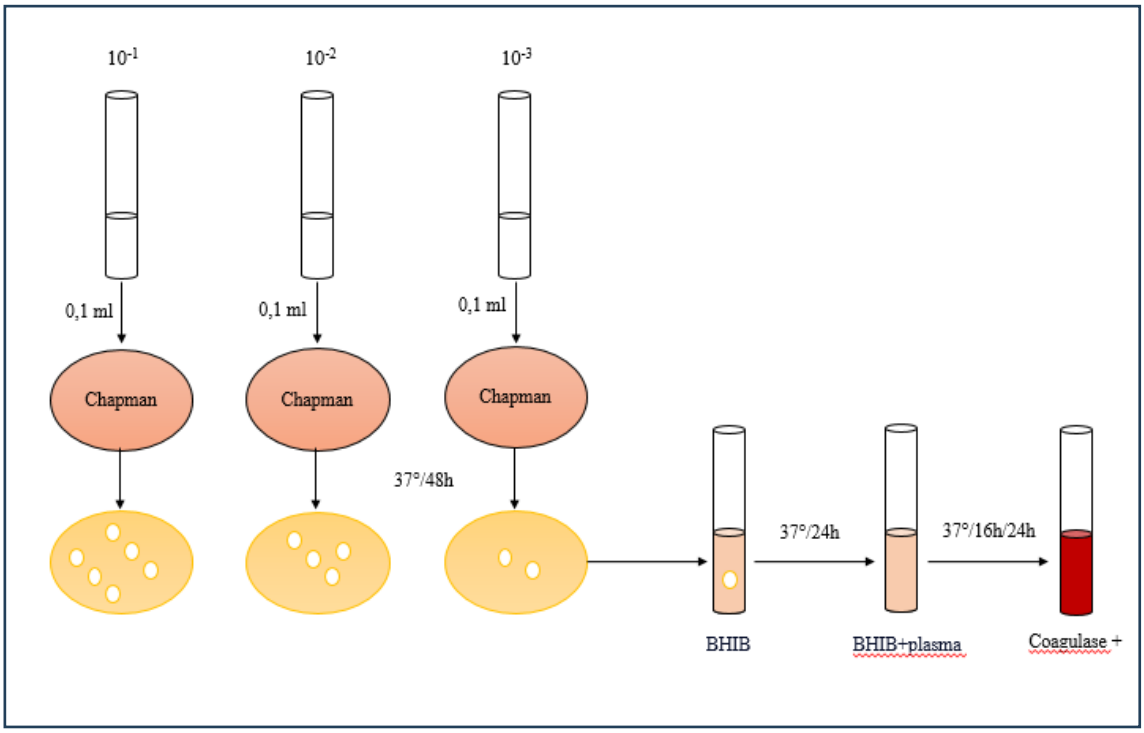


Figure 9: Enumeration of Coagulase-positive Staphylococcus

2.5.4. Enumeration of *Salmonella*

- **Purpose**

Salmonella is a pathogenic bacteria that can cause gastrointestinal infections in humans (Hendriksen *et al*., 2011), Salmonella continues to be a major concern for the dairy industry because it has caused outbreaks of illness (El-Gazzar and Marth, 1992), and nevertheless, adequate milk pasteurization inactivates this pathogen (Lobacz and Zulewska, 2021). Appropriate hygienic procedures during processing should reduce the likelihood of salmonellosis outbreaks associated with dairy foods (El-Gazzar and Marth, 1992).

- **Principe**

These bacteria are pathogenic leading to the preparation of a culture medium allowing the detection of colonies likely to be salmonella. The medium would be more selective concentration, among these media: SS agar (Salmonella, Shigella) (Dabboussi *et al*., 2013).

➢ **Procedure**

1. Pre-enrichment

25 g of the sample was weighed and placed in a sterile plastic bag with 225 ml of peptone water. The mixture was mixed vigorously for 2 minutes, then it was left for 10 minutes.

2. Enrichment

1 ml of the mother solution was transferred to a tube containing 9 ml of Rappaport-Vassiliadis enrichment broth (RV) and incubated at 37°C for 24 h.

3. Isolation

It was done on SS Agar (*Salmonella Shigella*) and incubated at 37° C for 24 hours.

4. Reading results

The SS agar dishes were read considering that Salmonella most often appears colorless with or without a black center (Dabboussi *et al*., 2013).

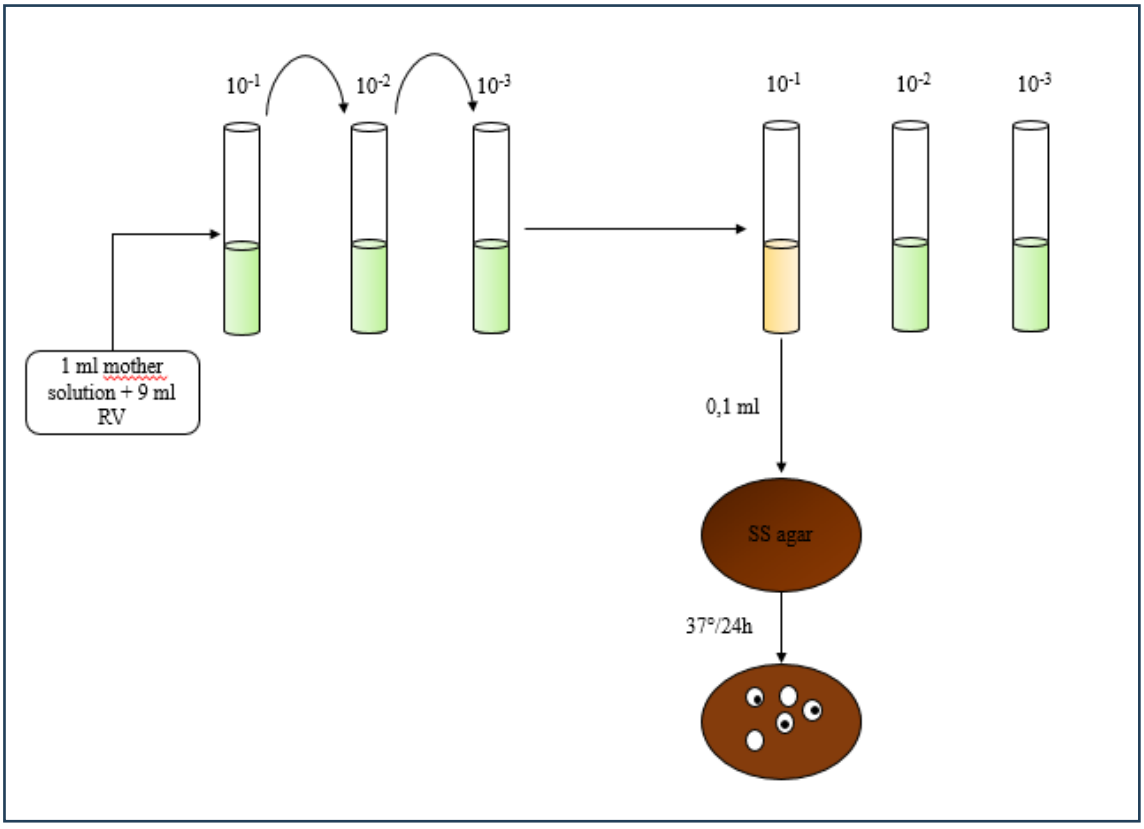


Figure 10: *Salmonella* Detection

2.5.5. Enumeration of lactic Acid Bacteria (LAB)

- **Purpose**

LAB has a long and safe history of application and consumption in cheese processing, thus being generally regarded as safe (GRAS) and leading to improvement in texture, flavor, and nutritional value (Kongo, 2013). A large amount of LAB, which are also present in fermented dairy products can be classified as probiotics (Coelho *et al*., 2022). LAB is important also in cheese processing because, it increases food safety through the release of lactic acid and bacteriocins (Kongo, 2013).

- **Procedure**

In reduced aerobic conditions, lactic acid bacteria in cheese samples were screened on an MRS medium (Figure 11) and incubated at 35°C for 48 h (Eljagmani and Altuner, 2020).

- **Reading results**

-The MRS agar dishes were read considering that LAB most often appears as off-white colonies.

-Individual colonies were tested for catalase reaction, gram reaction, and cell morphology (Figure 10).

-Gram-positive, catalase-negative (catalase activity by transferring an individual colony onto a glass slide and adding a drop of 3% (v/v) H2O2, cocci, or rods were tentatively considered to be lactic acid bacteria (Aprea et al., 2021).

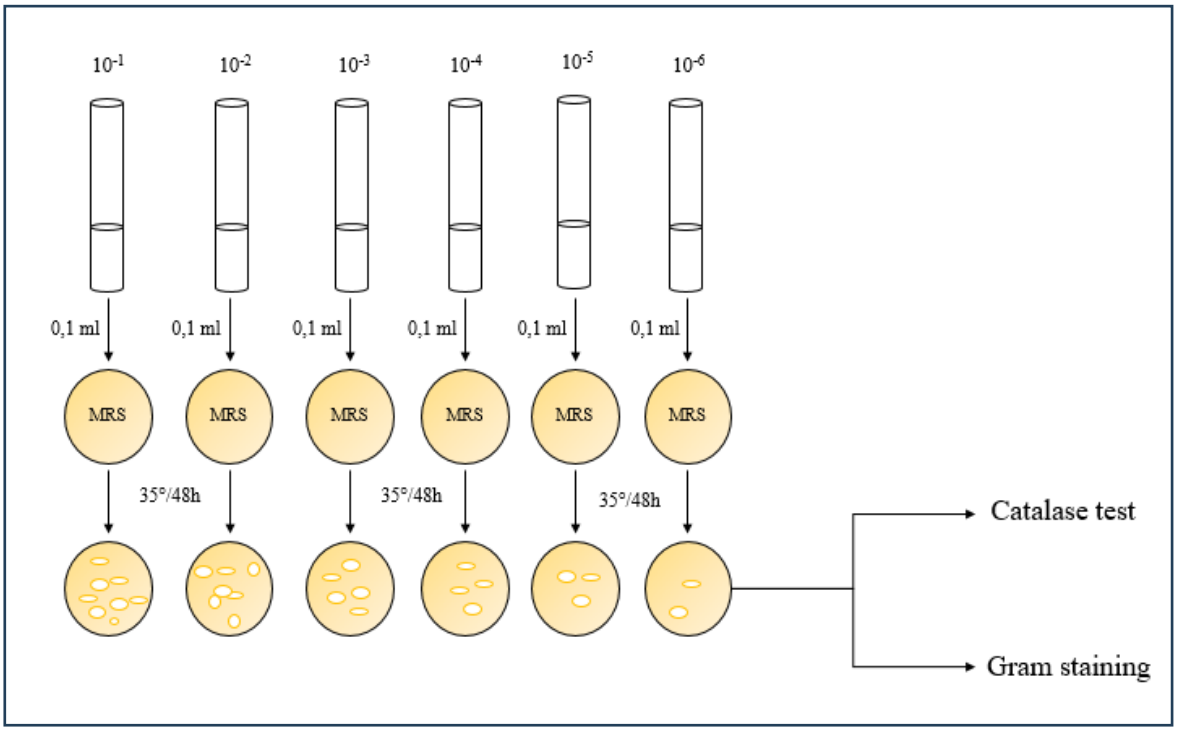


Figure 11: Enumeration and analysis of LAB

2.6. Statistical Analysis

To meet the objective of this study, a statistical analysis was carried out using Excel software (Microsoft Office, 2013). The data was prepared and imported into Excel, where different statistical functions were used to analyze the results.

CHAPTER 03

RESULTS AND DISCUSSION

3.1. Proximate Analysis

The proximate compositions of cheese samples are showed in Table 2.

Table 2: Mean values for the proximate composition of cheese samples

Samples	Moisture (%)	Ash (%)	Fat (%)
CC	79.1 ±0.4	0.5 ±0.6	4.0 ±1.6
PPC	43.6 ±0.4	1.3 ±0.6	22.7 ±1.6
AC	72.4 ±0.4	2.2 ±0.6	17.6 ±1.6

Data are expressed as means of duplicate determinations.
CC: cottage cheese; PPC: pasteurized processed cheese; AC: analogue cheese.

The mean moisture values found among all samples ranged from 43.6% to 79.1%, in which the CC sample had the highest content (79.1%), followed by the AC sample (72.4%), then the PPC sample with the lowest content of 43.6%.

The moisture content of the CC sample was 79.1%, which agreed with those reported by the United States Department of Agriculture (USDA, 2016) and the International Dairy Federation (IDF, 2004). The high moisture value of the CC sample was due to the curdling process of cottage cheese which uses rennet for initial curdling, but the process stops earlier. The curds are then separated further from the whey. This process retains more whey in the final product, leading to a higher moisture content (Wallas, 2021).

Generally, cottage cheese is made from skim milk, which naturally has a lower fat content than whole milk. The curdling process still results in a significant portion of the fat being separated in the whey, contributing to the higher moisture content (USDA, 2016). Also, Benedict and Ellis (1987) found that the fat content is inversely proportional to moisture.

The moisture value obtained from the PPC sample was 43.6% which was compatible with the moisture content reported by USDA (2016) ranging from 40% to 50%. The lower moisture content of PPC is due to the sum of various cheeses added, which often have lower moisture content, and the addition of emulsifying salts. This process allows for some moisture control. In addition, PPC produced with a specific texture and functionality and a moisture content of 40-50% strikes a balance between spreadability (high moisture can make the product too runny and difficult to spread), sliceability (low moisture can make the cheese dry, crumbly, and difficult to slice), and meltability (processed cheese within this range generally melts smoothly) (McGee, 2007).

In the case of the AC sample, the moisture content of the analogue cheese sample (72.4%) aligns with previous research on similar products. Studies by Scribner (2004) and Farmani et al. (2007) found that the moisture content of analogue cheese can range from 60 to 80%, depending on the specific recipe. This variation reflects the fact that different manufacturers may use different ingredients and production processes. This variation is attributed to the use of various plant-based ingredients (starches, nuts, oils, and vegetable proteins) (Gates, 2022).

Gates (2022) additionally reported that analogue cheese can have some flexibility, unlike traditional cheese where fat content and moisture content have an inverse relationship, the fat content can be adjusted (using plant-based fats or oils) to a certain extent, while still achieving a desired moisture content within the 60-80% range which allows for a spreadable or sliceable consistency similar to some soft and semi-hard cheeses.

Ash values were obtained in ascending order as follows 0.5%, 1.3 %, and 2.2 % for AC, PPC, and CC samples respectively. The ash content of the CC sample (0.5%) was similar to this mentioned by USDA (1999) which was from 0.4% to 0.6%. The obtained

value was in agreement with the value declared by IDF (1999) which is 0.5%, according to Tamime and Robinson (1999), the low ash content of CC was due to the whey separation process that removes a significant portion of the minerals along with the whey, resulting in a final product with a lower ash content.

The PPC sample had an ash content of 1.3%, which was relatively close to this reported by USDA (2004) which declared an ash content range of around 2-3% for processed cheese. PPC generally has a slightly higher ash content compared to CC, this was because PPC wasn't made solely from fresh curds like cottage cheese. It typically involves a blend of various cheeses, sometimes including natural cheeses with a higher inherent mineral content. Additionally, PPC contains added emulsifying salts (sodium citrate, phosphates) that contribute to the overall ash content (Lucey, 2004). Also, Gates (2022) noted that processed cheese often involves minimal whey removal, unlike cottage cheese production, this can lead to a higher concentration of minerals, reflected in the increased ash content.

In comparison with the other samples, the AC sample had the highest ash content which was 2.2%, this was due to the fact that some analogue cheese was fortified with additional minerals like calcium, potassium, or phosphorus to enhance their nutritional profile (NIH, 2022). The recipe and ingredient choices can influence the final ash content (Gates, 2022).

The fat content results exhibited a clear contrast in which CC has the lowest fat content of 4%, followed by the AC sample which had 17.6%, and the lowest fat content was for PPC with 22.7%. The CC's fat content of 4% was within the range allowed by USDA (2022) and IDF (2022) for low-fat cottage cheese which is recommended to be around 3-5%, this is due to the type of milk used which naturally has a lower fat content compared to whole milk used in some processed and analogue cheeses. However, modern varieties may

use whole milk. Even in this case, the curdling process efficiently removes a substantial amount of fat, resulting in a low-fat final product (Gates, 2022).

The PPC sample with a fat content of 22.7% was in agreement with McSweeney *et al*. (2018) who said that the fat content of processed cheese can vary depending on the type and amount of cheese used but typically falls within a range of 20% to 30%. The finding result was relatively close to those declared by Bachman (2001) who noted that the processed cheese fat content ranged from 25% to 30%.

According to Fox *et al.* (2017), PPC often combines various cheeses (ex. cheddar cheese) some of which may naturally be higher in fat content compared to skim milk used in cottage cheese production, furthermore, during the processing of PPC butter or cream might be added to the cheese mixture which leads to a further elevates of the overall fat content of the final product. Also, it plays a critical role in the texture of processed cheese, contributing to a smooth, spreadable consistency. 20% to 30% range allowed to create a lower-fat version for health-conscious consumers while still maintaining some of the desirable qualities associated with fat (McSweeney *et al*., 2018).

The fat content of analogue cheese was 17.6% this was due to the diversity of the ingredients (like nuts, seeds, starches, and vegetable oils) in comparison with cottage cheese which has a more standardized production process, the addition, these ingredients which have varying fat contents can contribute more fat to the final product to achieve a desired fat range, often targeting a middle ground between low-fat and full-fat cheeses (Bachmann, 2001; Fox *et al*., 2017). Marangoni and Barbosa (2008) developed a low-fat analogue cheese, they achieved a spreadable texture with a fat content as low as 15%, indicating that 17.6% fat falls within a reasonable range for some analogue cheese varieties (Marangoni and Barbosa, 2008).

3.2. Physico-chemical analysis

3.2.1. pH determination

The mean pH values of CC, PPC, and AC samples are shown in Figure 12. The obtained values showed a significant difference between the CC, PPC, and AC samples, in which the CC sample had the lowest value of 4.93 while the PPC and AC samples had the same value of 6.38.

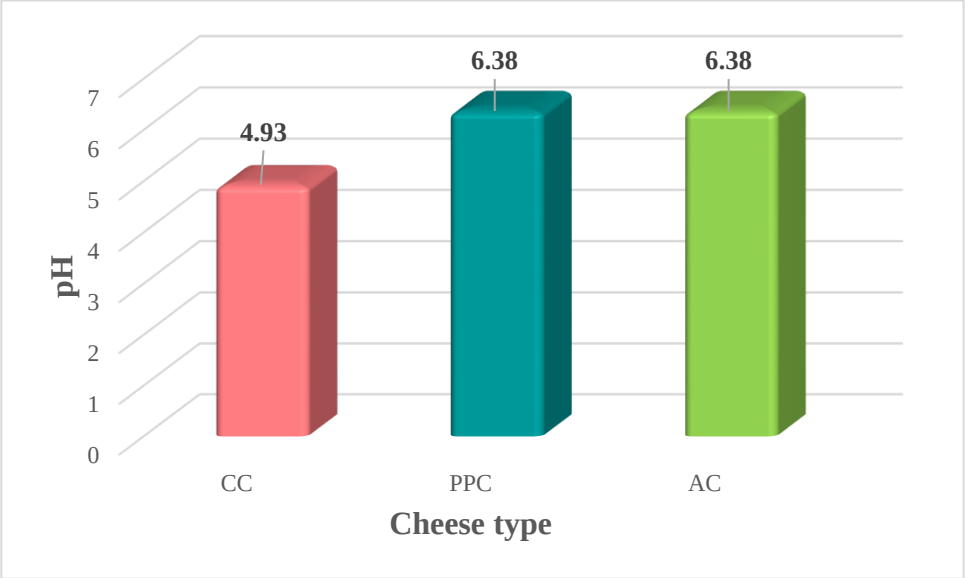


Figure 12: Mean values of pH for cottage cheese (CC), pasteurized processed cheese (PPC), and analogue cheese (AC)

The result of the CC sample (4.93) was in agreement with Yoon *et al.* (2008) and Tamime and Robinson (2007) who studied the pH of cottage cheese and reported that its pH ranged from 4.6 to 5.2, this may be explained by the action of lactic acid bacteria which is, as reported by Tamime and Robinson (2007), responsible for the fall in pH by producing lactic acid during fermentation.

The measured pH value obtained from the PPC sample (6.38) agreed with that reported by Fox *et al*. 2016, who stated a typical pH range of 5.2 to 6.0 for processed cheese. They explained the higher pH in PPC compared to the CC one by the attribution of the adding

emulsifying salts (citrates, phosphates) during processing, which act as buffers, raising the pH and contributing to a smoother texture.

In the case of the AC sample that had the same PPC's pH value (6.38), this founding result was similarly reported by Tamime and Robinson (2007), and they noted that in analogue cheese processes, some ingredients used like acidifying agents can influence its pH to achieve a similar pH range as processed cheese.

3.2.2. Determination of titratable acidity

The means titratable acidity expressed as a percentage of lactic acid of the cheese samples are shown in Figure 13. The obtained values were 0.15%, 0.12%, and 0.15% for CC, PPC, and AC respectively.

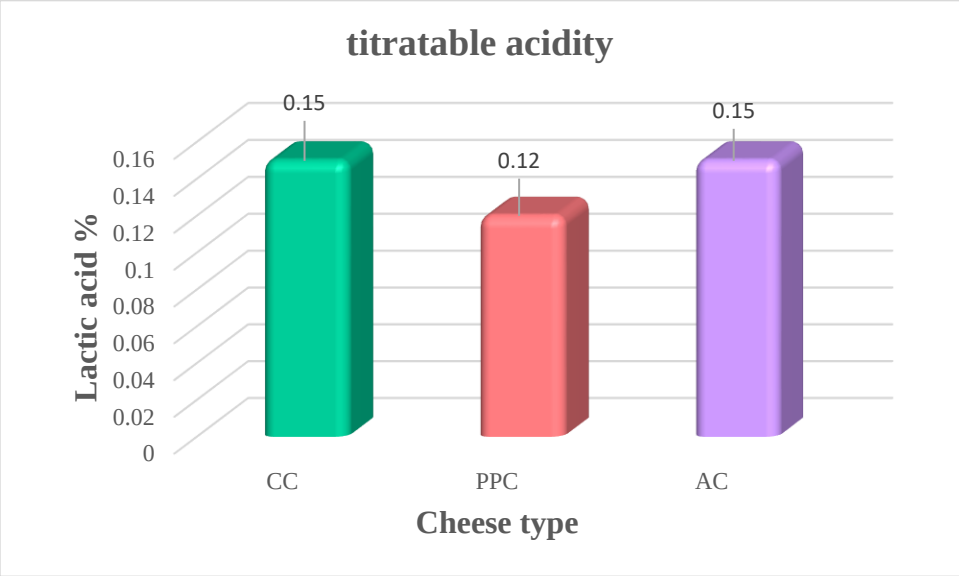


Figure 13: Mean values of titratable acidity of cottage cheese (CC), pasteurized processed cheese (PPC), and analogue cheese (AC)

The titratable acidity of CC was 0.15% which was in agreement with the findings of Fox et al. (2004), who reported a range of 0.12% to 0.18% lactic acid for cottage cheese, this was explained by the action of lactic acid bacteria used as starter cultures can ferment lactose at varying rates, leading to a range of lactic acid production and reflecting its fresh and

unripened nature. While, Tamime, (2007) noted a range of 0.4% to 0.6% for lactic acid in some cottage cheese varieties, which disagreed with the current study. The authors explained the obtained range by the potential influence of production methods including curd washing which removes some lactose and lactic acid, concluding that less washing leads to higher acidity. On the other hand, adding cream or other ingredients could dilute cheese's overall lactic acid concentration.

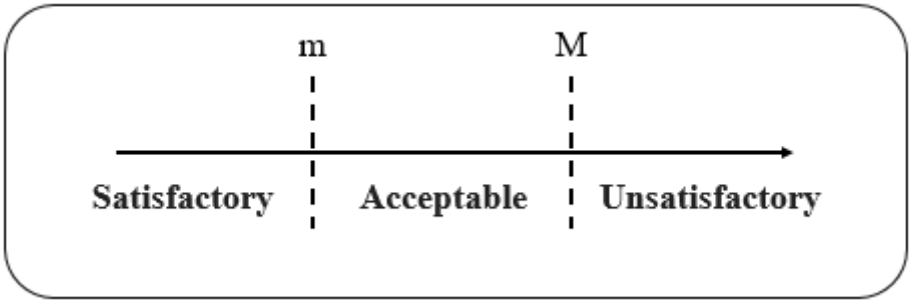
The lactic acid value of processed cheese reported by Jang et al. (2016) ranged from 0.30% to 0.42% which was in disagreement with the current study (0.12%), Jang et al. (2016) claiming that the type and amount of additional ingredients can significantly impact acidity level in processed cheese, where different cheeses used as a base in processed cheese can have varying lactic acid levels depending on their production methods. High-acid cheeses like cheddar will contribute more lactic acid, while mozzarella (lower acidity) will have a lesser impact. The authors added that processed cheese can include emulsifiers, stabilizers, and acids. The specific type and amount of these ingredients can significantly affect the overall acidity. For example, adding citric acid can increase acidity, while buffering agents might lower it.

The acidity value of the AC sample was 0.15%, a similar finding was reported by Fox *et al*. (2004), who found that cottage cheese acidity ranged from 0.12% to 0.18% of lactic acid. Lucey, (2011) pointed out that analogue cheeses don't undergo fermentation with lactic acid bacteria like the traditional ones, they added that analogue cheese achieves its acidity by adding organic acids, such as citric acid. Therefore, even though the acidity level of analogue cheese might be similar to the cottage but it is for different causes.

3.3. Microbiological Analysis

The significance of the microbiological results of cheese samples was evaluated according to the microbiological criteria established by the Official Journal of the Algerian Republic (JORA) in 2017, which uses a two or three-class plan. Microbiological sample criteria include four letters: n, c, m, and M, where:

- **n:** the number of units of the sample;
- **c:** the maximum number of sampling units that can exceed m while being greater than M without the lot being rejected;
- **m**: the number of germs present in a gram of product analyzed below which the quality of the product is considered satisfactory;
- **M:** the number of germs present in a gram of product analyzed above which the quality of the product is considered unacceptable. Using a three-class design, the sample is considered (JORA, 2017):
- **Satisfactory microbiological quality**: the result of the analysis is less than or equal to m.
- **Acceptable microbiological quality:** the result of the analysis does not exceed M and if the number of units of the sample gives a result greater than m between 1 and c.
- **Unsatisfactory microbiological quality:** the result of the analysis exceeds M or if the number of units of the sample giving a result between m and M is greater than c.



3.3.1. *Escherichia Coli*

Table 3 shows the results of the microbiological quality of cheese samples based on the microbial load of *E. coli* according to JORA, (2017) limits.

Table 3: *E. coli* enumeration results in analogue, processed, and cottage cheese

Germ	Sampling plan		Microbiological limits CFC/g (JORA, 2017)		Results		
	n	**c**	**m**	**M**	**AC**	**PPC**	**CC**
E.coli	1	2	10^2	10^3	0	0	0
	2				0	0	0
	3				0	0	$3.1*10^3$
	4				0	$6.3*10^2$	0
	5				0	$2.6*10^3$	0
					Microbiological quality		
					Satisfied	**Acceptable**	**Acceptable**

The AC sample showed a negative results, this can be due to the absence of source material since the analogue cheese wasn't made with animal products like milk. Also, the manufacturing process for analogue cheese involved strict hygiene protocols to prevent contamination from other sources by sterilizing of equipment used during production, using of filtered air and water to minimize bacterial contamination, and proper handling of ingredients by workers to prevent cross-contamination (IDF, 2011; FDA, 2017).

PPC sample showed a satisfied quality, this can be due to the processing that often involves high temperatures during cooking and emulsification stages. These high temperatures can further reduce or eliminate any remaining *E. coli* from the raw ingredients. Also the Processed cheese generally has a lower water activity (Aw), compared to other dairy products like milk or fresh cheese, in which *E. coli* prefers environments with higher water activity. The low (Aw) creates an unfavorable environment for *E. coli* to survive or multiply (Richard and Robinson, 2008; Rahman, 2020).

The results of *E.coli* in CC sample were consistent with JORA (2017), which indicates that the pasteurization of milk used for this cheese was successfully made by destroying the harmful bacteria, including *E. coli*. By using pasteurized milk, manufacturers significantly reduce the risk of *E. coli* contamination in the final product. It also can be due to addition of specific starter cultures like LAB, which ferment the lactose sugars in milk, producing lactic acid, this acidic environment inhibits the growth of *E. coli*, which generally prefer a neutral or slightly alkaline environment (Tamime and Robinson, 1991; CDC, 2020).

Contamination of cheese by *E. coli*, even though less common than in other foods, but can still pose a health risk. While many cheese varieties harbor harmless *E. coli* strains, some strains, like Shiga toxin-producing *E. coli* (STEC), can cause serious illness. STEC strains, like E. coli O157:H7, can contaminate cheese through improper handling of raw milk during production or from fecal contamination during aging. Consuming of contaminated cheese can lead to severe abdominal cramps, bloody diarrhea, and fever. In rare cases, complications like hemolytic uremic syndrome (HUS) can develop, affecting the kidneys and other organs. Choosing cheese made from pasteurized milk and practicing safe handling techniques can significantly reduce the risk of E. coli infection from cheese (CDC, 2023).

3.3.2. *Staphylococcus*

The Table 4 showed a satisfied microbiological quality for AC and CC samples while PPC one presented an acceptable quality. The sampling plan and the microbiological limits are based JORA (2017).

Table 4: *Staphylococus* enumeration results in analogue, processed, and cottage cheese

Germ	Sampling plan		Microbiological limits CFU/g (JORA, 2017)				Results		
			Analogue & Processed cheese		Cottage cheese				
	n	**c**	**m**	**M**	**m**	**M**	**AC**	**PPC**	**CC**
Staph	1	2	10^2	10^3	10	10^2	0	0	0
	2						0	0	0
	3						0	0	0
	4						0	$3.8*10^2$	0
	5						0	0	0
							Microbiological quality		
							Satisfied	**Acceptable**	**Satisfied**

AC sample gave a negative results for *Staphylococcus,* this could be due to the dependence on the specific processing steps, in which might involve high temperatures. While the exact temperatures needed to eliminate *Staphylococcus aureus* may vary, high temperatures can generally reduce bacterial populations. It also can be due to the rigorous hygiene protocols by the manufacturers in place throughout processing like proper sanitation of equipment and production environment to minimize bacterial presence (Richard and Robinson, 2008; IDF, 2011).

The microbiological quality of the PPC sample was acceptable, which could be due to the use of pasteurized milk or cheese products made from pasteurized milk, as a raw ingredient. Also, many processed cheeses contain ingredients like citric acid or lactic acid to enhance flavor and texture, this creates a slightly acidic environment that inhibits the growth of *Staphylococcus aureus*, which thrives in neutral or slightly alkaline conditions (Richard and Robinson, 2008; CDC, 2020).

The CC sample showed a satisfying quality. This could be due to the pasteurized milk used. Also, cottage cheese is typically stored in low temperatures, which further slows down the growth of any remaining *Staphylococcus aureus* bacteria. Refrigeration temperatures inhibit bacterial growth and reproduction (CDC, 2020; FDA, 2023).

Symptoms of staphylococcal food poisoning typically include nausea, vomiting, severe stomach cramps, and diarrhea. In severe cases, the toxins can lead to more serious complications like dehydration, low blood pressure, and even death. Therefore, proper food handling practices, such as maintaining good hand hygiene and cooking foods to safe internal temperatures, are essential to prevent *Staphylococcus aureus* contamination and the associated risk of foodborne illness (CDC, 2019).

3.3.*3 Salmonella*

The tests for *Salmonella* detection showed a negative results in all the cheese samples (Table 5).

Table 5: *Salmonella* determination in analogue, processed, and cottage cheese

Germ	Sampling plan		Microbiological limits CFU/g (JORA, 2017)	Results		
	n	**c**		**AC**	**PPC**	**CC**
Salmonella	1	0	Absence in 25g	Absence	Absence	Absence
	2					
	3					
	4					
	5					
				Microbiological quality		
				Satisfied	**Satisfied**	**Satisfied**

The obtained result of AC due to the absence of the initial source, *Salmonella* is commonly found in the intestines of animals and can contaminate raw milk. Since analogue cheese doesn't contain animal-derived ingredients, it eliminates the primary source of *Salmonella*. Moreover the high temperatures used during processing, reduce the bacterial load (Richard and Robinson, 2008; IDF, 2010).

The absence of salmonella in the PPC sample owing to the acidic environment because many processed cheeses incorporate ingredients (citric acid or lactic acid). Furthermore the PPC often contains additional ingredients that bind water and reduce the overall water activity (Aw) of the product. Salmonella bacteria require a certain level of

moisture for growth and survival. The reduced water activity in processed cheese makes it a less favorable environment (Shafiur and Theodore, 2007; Richard and Robinson, 2008).

In the case of the CC sample, the obtained result was because of the pasteurized milk used in the manufacturing of this type of cheese and LAB action by lowering the pH and creating an acidic environment (Tamime and Robinson, 1991; CDC, 2020).

Salmonella contamination in food poses a significant danger to human health. Consuming food containing *Salmonella* bacteria can lead to salmonellosis, a form of foodborne illness causing a variety of unpleasant and potentially severe symptoms (diarrhea, abdominal cramps, fever, nausea, and vomiting). In severe cases, *Salmonella* can lead to dehydration, which requires medical attention. Young children, pregnant women, and older adults are more susceptible to serious complications from *Salmonella* intoxication. Safe food handling techniques and proper hygiene are crucial to prevent contamination and safeguard human health **(**CDC, 2023).

3.3.4. Lactic acid bacteria (LAB)

The LAB loads for AC, PPC, and CC samples were 0 CFU/g, $4.5*10^5$ CFU/g, and $5.6*10^7$ CFU/g respectively, in which the LAB count of the CC sample was the highest (Table 6).

Table 6: Lactic acid bacteria (LAB) mean values (CFU/g) of the cheese samples with catalase and gram tests

Germ	**AC**	**PPC**	**CC**
LAB	0	$4.5*10^5$	$5.6*10^7$
Tests		**Catalase**	
	/	Positive	Positive
		Gram coloration	
	/	Positive	Positive

Data are expressed as means of duplicate determinations.
CC: cottage cheese; PPC: pasteurized processed cheese; AC: analogue cheese.

The absence of LAB in the AC sample was because of the absence of dairy products. Traditional cheesemaking relies on LAB cultures, but analogue cheese misses milk altogether. This eliminates the need for LAB and their role in texture and flavor development. Instead, analogue cheese uses alternative ingredients like plant-based oils and starches (Tamime, 2007).

Compared to the CC sample, PPC had a lower LAB load. Fabrication of processed cheeses prioritizes factors like texture, meltability, and flavor, that's why starter cultures and their final count might be less emphasized. Even if no additional LAB is introduced during processing, some residual LAB from the types of cheese used as base ingredients might be present in the final product. The remaining LAB may play a role in flavor development and shelf life extension, but their quantity might not be as crucial as in the case of cottage cheese (Richard and Robinson, 2008).

Cottage cheese production relies on starter cultures containing specific LAB strains. These cultures are introduced to the milk to ferment the present lactose, this process contributes to the characteristic mild acidity and smooth texture of cottage cheese. Lactic acid production by LAB also helps preserve cottage cheese by creating an acidic environment that inhibits the growth of undesirable bacteria (Tamime, 2007).

The LAB under the microscope seem as gram-positive, cocci, coccobacilli, or rods. After confirmation with biochemical tests, the confirmed tests included (Gram stain, and catalase) it was gram positive, catalase positive.

Lactic acid bacteria (LAB) are a welcome presence in many cheeses, offering a variety of benefits throughout the production and consumption process. One key advantage is their role in texture and flavor development in cheese (Tamime, 2007).

CONCLUSION
AND
RECOMMENDATIONS

Conclusion

The present research was designed to compare the characteristics of cottage cheese, pasteurized processed cheese, and analogue cheese. The results obtained from this study showed that the CC sample had higher moisture content but lower ash and fat contents in comparison with PPC and AC samples, due to the ingredients and the process used during manufacturing for technological reasons.

The pH values of all the cheese samples were acceptable and within the cheese quality criteria, in which the mean pH values of CC samples were significantly lower, owing to the action of LAB, in comparison with those of PPC and AC samples which had the same pH value due to the added emulsifying salts and acidifying agents respectively. The mean values of titratable acidity of the CC sample and the AC sample were similar but for different reasons, LAB action and added organic acids respectively, the PPC sample had the lower value due to the type and the amount of the ingredients used.

All samples showed high microbial quality where *S. aureus*, *E. coli*, and *Salmonella* were within the JORA standards. In the case of LAB, the CC sample had the highest load because LAB was added as a fermentation agent in comparison with PPC which had some residual LAB from the types of cheese used as base ingredients, while the AC sample had a negative result due to the absence of dairy products.

Recommendations

- The present study can be further expanded by doing the protein dosage as well as the types of amino acids present in these foodstuffs.
- This work is far from complete and must be supplemented by the determination of vitamins and minerals matter, and the extent of lipid oxidation of the product to get more information on the effect of processing and storage on the quality of lipids.
- Further research has to be carried out on the fatty acid profile of the analyzed cheese to have more information from a nutritional point of view.
- It is recommended to do the enumeration of *Listeria monocytogenes* according to JORA standards

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APPENDIX

Appendix 1: Questionnaire used to collect data based on the most consumed cheese among people in Guelma City:

In the context of preparing scientific research to obtain a Master's degree in Quality of Products and Food Safety, the research involves a comparative study of selected cheese varieties, we request that you complete this questionnaire with care.

Your responses will remain anonymous and confidential.

1. Gender

Men ☐

Women ☐

2. Age

18 - 35 years ☐

36 - 45 years ☐

46 - 55 years ☐

56 - 66 years ☐

> 66 years ☐

3. Level of education:

Primary ☐

Secondary ☐

Higher ☐

Other ☐

4. Professional activity:

Student ☐

Employee ☐

Unemployed ☐

Other ☐

5. Do you eat cheese?

Yes ☐

No ☐

6. Frequency of cheese consumption

Daily ☐

1- 2 times per week ☐

> 3 times per week ☐

1 - 2 times per month ☐

> 3 times per month ☐

Rarly ☐

7. What is your favorite cheese?

Processed cheese ☐

Mozzarella ☐

Analogue cheese ☐

Cottage cheese ☐

Camembert ☐

Other (please specify)

……..

8. What is the criterion that influences your choice for this type of cheese?

Flavor ☐

Price ☐

Nutritional value ☐

Fat content ☐

Availability ☐

Out of habit ☐

Texture and Consistency ☐

Culinary Application ☐

Autre (veuillez préciser)

……

9. Have you ever experienced food poisoning after consuming cheese?

Yes ☐

No ☐

Appendix 2: Journal Officiel de la République Algérienne N° 39. juillet 2017

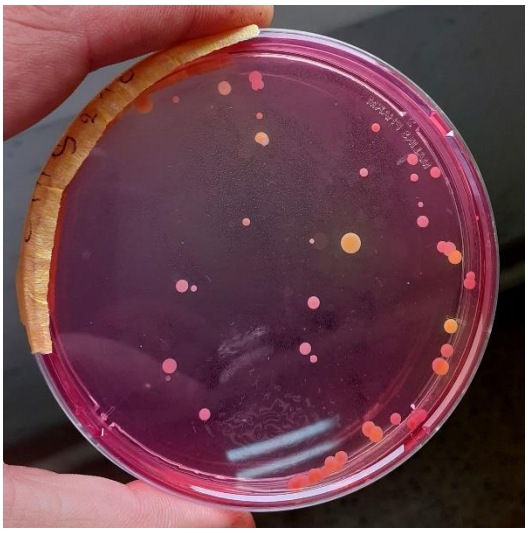
Fromages à base de lait ayant subi un traitement thermique moins fort que la pasteurisation et fromages affinés à base de lait ou de lactosérum pasteurisés ou ayant subi un traitement thermique plus fort que la pasteurisation	*Escherichia coli*	5	2	10^2	10^3
	Staphylocoques à coagulase +	5	2	10^2	10^3
	Salmonella	5	0	Absence dans 25 g	
	Listeria monocytogenes	5	0	100	
Fromages à pâte molle non affinés (fromages frais) à base de lait ou de lactosérum pasteurisés ou ayant subi un traitement thermique plus fort que la pasteurisation	*Escherichia coli*	5	2	10^2	10^3
	Staphylocoques à coagulase +	5	2	10	10^2
	Salmonella	5	0	Absence dans 25 g	
	Listeria monocytogenes	5	0	100	

Appendix 3: Photos of the process of work

Colonies of E.coli

(Aliouche ahmed rami

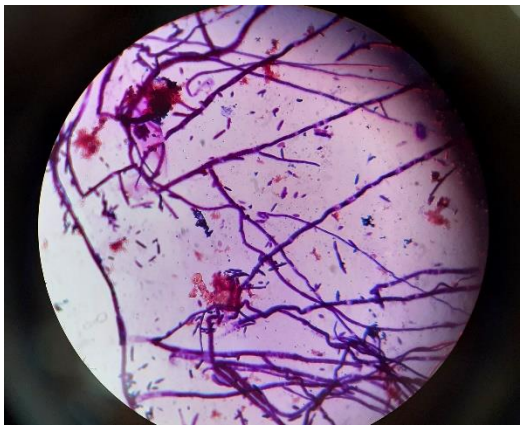
Maalem Thabet , 2024)



Colonies of Staph

(Aliouche ahmed rami

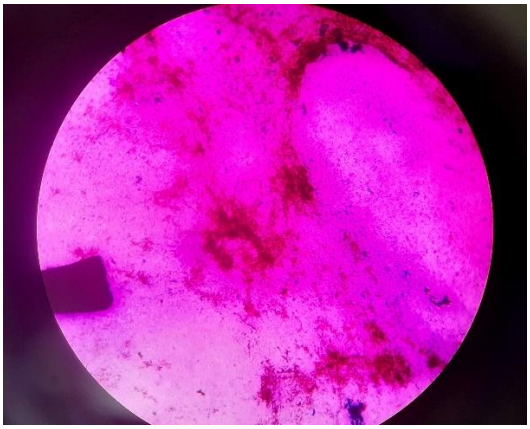
Maalem Thabet , 2024)



Gram-positive for processed cheese

(Aliouche ahmed rami

Maalem Thabet , , 2024)

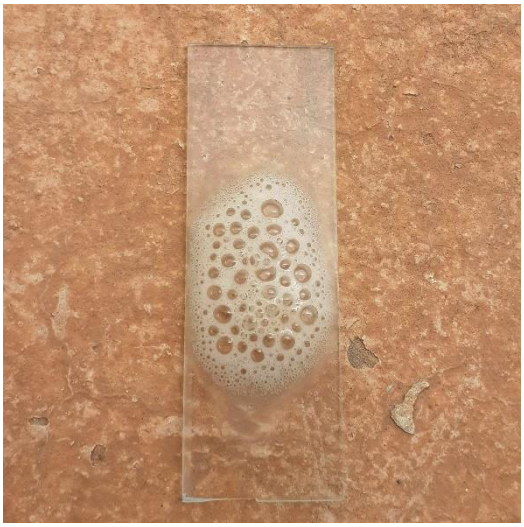


Gram-positive for cottage cheese

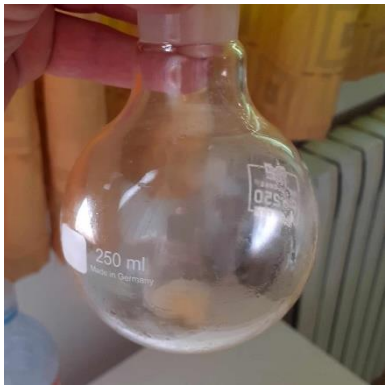
(Aliouche ahmed rami

Maalem Thabet , , 2024)

Catalase positive for processed cheese (Aliouche ahmed rami Maalem Thabet , 2024)



Catalase positive for cottage cheese (Aliouche ahmed rami Maalem Thabet , 2024)



Fat Extraction in analogue, processed, and cottage cheese (Aliouche Ahmed Rami, Maalem Thabet ,2024).

Work in university laboratory (Aliouche ahmed rami, Maalem Thabet 2024)