SELINUS UNIVERSITY
OF SCIENCES AND LITERATURE

NUTRITIONAL AND MICROBIOLOGICAL QUALITY OF RAW COW'S MILK SOLD AT THE BAGNON MARKET (ABIDJAN-YOPOUGON), COTE D'IVOIRE

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A DISSERTATION

Presented to the Department of
Food Science
program at Selinus University

Faculty of Natural Health Science
in fulfillment of the requirements
for the degree of
Doctor of Philosophy in Food Science

2023

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ABSTRACT

Nutritional and microbiological quality of raw cow's milk sold at the Bagnon Market (Abidjan-Yopougon), Côte d'Ivoire (Ivory Coast)

Pathogenic microorganisms may be present in milk, from sick cow or from contamination by handlers, *Streptococcus* causing udder infection (mastitis), *Brucella* (Brucellosis), *Mycobacterium bovis* and *tuberculosis* (Tuberculosis) are examples.

The milk thus obtained at milking and sold as is, or only refrigerated, is raw milk.

In reality, milk is rarely sold raw, its conservation being extremely limited: it undergoes treatments (skimming, heating, processing technology, etc.).

However, in Yopougon (the largest municipality among the ten ones in Abidjan, our economic and administrative capital town), many people prefer to consume the raw cow's milk sold at the market (report from the town hall). They give as an argument to preserve all the nutritional qualities of raw milk that the heat would degrade.

There is local consumption; of course, we also want to produce milk locally to avoid the high import costs. We want to check adulteration with water, preservatives, added solids such as addition of starch, etc.

Thus, the first objective focused on the literature review which deals with the nutritional importance of milk and the health risks due to the proliferation of microorganisms in raw cow's milk.

The second objective dealed with the results of our study followed by discussion in order to propose more efficient milking methods for the consumption of nutritious and safe raw cow's milk, according to the requirements of the *Codex Alimentarius*.

According to the *Codex alimentarius*, a good quality of raw milk has to be free of debris and sediment, free of off-flavors and abnormal color and odor, chemicals (e.g., antibiotics, detergents); low in bacterial count, must have a normal nutritional composition.

Our raw milk is certainly free of debris and sediment, off-flavors; abnormal color and odor. However, it had not a good quality because it met no good microbiological quality.

The quality of our raw cow's milk could be improved by using good hygiene practices by minimizing health risks related to Milk, Men, Methods, Material and Milieu during milking.

LIST OF ABBREVIATIONS

AARB: Alcohol-Acid-Resistant Bacteria
BPT: Buffered peptone water
BRC: British Retail Consortium
CFU: Colony forming unity
COR: Corrupt
°C: Degree Celsius (**°C** × 9/5) + 32 = **°F**
°F: Degree Fahrenheit (°F- 32) × 5/9 = **°C**
EHEC: Enterohaemorragic Escherichia coli
EIEC: Enteroinvasive Escherichia coli
EPEC: Enteropathogenic Escherichia coli
ETEC: Enterotoxigenic Escherichia coli
FAO: Food Agriculture Organization
FSSC: Food Safety System Certification
GC: Gas chromatography
GFSI: Global Food Safety Initiation
HACCP: Hazard Analytical Critical Control Points
HPLC: High Performance Liquid Chromatography
IFS: International Featured Standard
MAGs: Mesophilic Aerobic Germs
MAX: Maximum
n: Dilution exponent
N: Number of Germs
NA: Nutrient Agar
OD: Optic density (absorbance)
PCR: Polymerase chain Reaction
PH: Potential Hydrogen
SAT: Satisfactory
SS: Stock Solution
TACCP: Threat Assessment and Critical Control Points
VACCP: Vulnerability assessment and Critical Control Points
WHO: World Health Organization

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INTRODUCTION

A universal problem has preoccupied the times since the origin of men: how to maintain life? Life expectancy has ranged from 900 to 70 years old **[32].**

Man is therefore faced with the problem of life and death. The primary objective is and has been: how to last as long as possible? It will be reflected in all human activities. Man wants to extend his life but also to live in the best possible conditions. Man's first target will be to fight death.

Death is usually caused by disease, so we must fight the causes (biological, chemical and other factors) to increase life expectancy, and therefore health.

Health is a state of physical, social, mental, moral and spiritual well-being. A person is said to be in good health if he is able to meet those basic needs **[12].**

The quality of food is therefore a first criterion for evaluating human health. We must eat balanced, for example, without being contaminated especially by biological agents.

So, if there is one area where quality control is a fundamental necessity, it is that of food products in general and milk in particular.

At all stages, from the raw material to the finished product, special attention must be paid to that aspect.

Among the factors that can influence the quality of milk and milk products, microbial agents play a predominant role, whether it is the "useful microbial flora" that are involved in the manufacture of cheese, yogurt, etc., or “useless microbial flora” like pathogenic bacteria **[25].**

Moreover, the raw cow's milk sold at the Bagnon market is obtained as follows:

- Cows are naturally and occasionally fed with cassava skins, wild grasses by the shepherds.
- Cows that have to be milked are tied up by the hind legs to be immobilized. The calves will be suckled on the first spray for about 10 minutes every morning between 6 and 7 o’clock. The milking is then done manually in the open air (please see **Annex A1**) until a bucket of about 5 liters is filled, previously cleaned and washed with soap and water. The milking operation is repeated with a second bucket if necessary.
- The raw milk obtained is packaged in cans of approximately 10 liters for delivery and sale to vendors (wholesale buyers) at the Bagnon market (please see **Annex A1**) at the same purchase price (1liter/Euro). Since it is the same price at the milking and at the

market, customers prefer to buy it at the market, as the place of the milking is relatively far from the market (about 20 kilometers).

The milking technique does not make it possible to obtain sterile milk. The udder of the cow cannot be easily and reliably sterilized, nor the containers. However, contamination can be limited by obvious hygiene measures (cleanliness).

On the other hand, milk is an excellent culture medium, very "enriched". It is made up of water, lactose, lipids, proteins, mineral ions, vitamins. Its pH is close to 7 **[22].**

Clean raw milk from a healthy animal usually contains less than 1000 microorganisms per cm^3. They are lactic streptococci (*Lactococcus*), *Lactobacillus*, sometimes *Micrococcus*, commensals of the udder.

Pathogenic microorganisms may be present in milk, from sick cow or from contamination by handlers, *Streptococcus* causing udder infection (mastitis), *Brucella* (Brucellosis), *Mycobacterium bovis* and *tuberculosis* (Tuberculosis) are examples.

The milk thus obtained at milking and sold as is, or only refrigerated, is raw milk. In reality, milk is rarely sold raw, its conservation being extremely limited: it undergoes treatments (skimming, heating, processing technology, etc.) **[22].**

However, in Yopougon (the largest municipality among the ten ones in Abidjan, our economic and administrative capital town), many people prefer to consume the raw cow's milk sold at the market (report from the town hall). They give as an argument to preserve all the nutritional qualities of raw milk that the heat would degrade.

There is local consumption; of course, we also want to produce milk locally to avoid the high import costs. We want to check adulteration with water, preservatives, added solids such as addition of starch, etc.

We therefore want to know whether the raw cow's milk sold at the Bagnon market could not be responsible for food poisoning even if its nutritional qualities are affected or not.

Talking about food poisoning, WHO estimates that of the billions of people who travel each year, 20 to 50 percent suffer from foodborne illness **[11].**

In addition, a Canadian study carried out in 1982 established that out of 1037 cases of food poisoning, 982 cases were of microbial or biological origin, or 94.7% **[30].**

Thus, over the past fifteen years, FAO has collaborated with several different parts of the world to assess the quality, safety and socio-economic aspect of street food and to implement recommendations aimed at improving them **[14].**

Meat from mammals and poultry and their by-products are responsible for epidemic food poisoning in 70% of cases. Other foods (fish and other seafood, eggs, milk and dairy products) are responsible in 20% of cases **[30].**

In 2001, around 42% of food products were non-compliant from a hygienic quality point of view **[24].**

In Côte d'Ivoire, there have been an increasing number of foodborne illnesses (typhoid fever, cholera, etc.) over the past twenty years. They were reinforced by the consequences of the war of September 19, 2002: decomposing human corpses during the rainy season, displacement of populations from war zones to Abidjan favoring epidemics, etc. **[24].**

In the last ten years, in Côte d'Ivoire, cases of food poisoning have also been revealed: Hôtel Ivoire, Sococé, Agnibilékrou, Bodokro, etc. **[24].**

To curb these social dangers, the Consumers Union has proposed repression in collective catering, in street food in all the municipalities of Côte d'Ivoire in the event of poor hygiene.

But the government has instead proposed that we do it instead of repression, awareness-raising and training.

So, our thesis subject is: **"Nutritional and microbiological quality of raw cow's milk sold at the Bagnon market (Abidjan-Yopougon), Côte d'Ivoire (Ivory Coast)".**

The objective of this work is to:

- Explain the nutritional importance of milk;
- Evaluate the toxic potential of raw milk by microbiological analyzes;
- Propose more efficient milking methods to consume raw milk without great danger.

Our work will therefore consist of three parts:

- The first part will focus on the literature review which deals with the nutritional importance of milk and the health risks due to the proliferation of microorganisms in raw cow's milk.
- The second part will be devoted to the study itself which will outline our study methodology.
- The third part will deal with our results followed by discussion in order to propose more efficient milking methods for the consumption of nutritious and safe raw cow's milk, according to the requirements of Codex Alimentarius.

I. FIRST PART: LITERATURE

I.1. Quality of cow's milk

I.1.1. Definition of milk

Milk is a white, opaque liquid with a slightly sweet flavor, constituting a complete and balanced food, secreted by the mammary glands of women and by those of female mammals for the nutrition of young people **[2] [4].** The *Codex Alimentarius* (*CODEX STAN 206-1999*) defines it as "the normal mammary secretion of milking animals obtained from one or more milkings, without adding or subtracting anything, intended for consumption as liquid milk or further processing".

I.1.2. Organoleptic characteristics [8]

Organoleptic properties are the aspects of food or other substances as experienced by the senses, including taste, sight, smell, and touch. For raw cow's milk, it is: appearance and texture (liquid opaque to light), odor (odors related to fat), color (pearly white color), density (1.032 to 25°C), pH (6.7 +/- 0.1), Dornic acidity (16-18°), viscosity (resistance of milk to flow, the viscosity of raw cow's milk is: 0.02 Ns/m^2), etc.

I.1.2. Quality control of milk [20].

The quality of a product is defined as the set of characteristics enabling it to meet the needs expressed by consumers. The quality of milk and dairy products derived from it, is a concept with many facets.

Physical aspects	Freezing point, volumed mass, color, fat separation, specific heat, viscosity, etc.
Chemical aspects	PH, acidity, antibiotics, proteins content, lipids, lactose, minerals, etc.
Microbiological aspects	Bacteria, somatic cells, viruses,
Conservation properties	Microbial flora, enzymes, oxygens, etc.
Functional properties	Heat stability, rennet coagulation, emulsification, expansion, etc.
Bifunctional properties	Nutritional value (vitamins, minerals, Omega-3, probiotics, content, etc.); enzymatic fermentation and hydrolysis (bioactive peptides, hydrolyzed lactose, etc.).

Table I.1. Various facets of milk quality

The one we hear most often is undoubtedly the microbiological quality which is directly related to the safety of milk, which is not surprising since it generally has a direct and very short-term impact on the health of the milk consumers.

Despite all the nuances that we would like to bring to the notion of milk quality, no one will dispute that the notion of harmlessness remains central. If one accepts a broad definition of safety as "the quality or character of something which is not harmful or toxic', then the safety of milk refers to the fact that it will not render sick the consumer. It must be agreed that in this matter, it is above all the chemical and microbiological aspects that should receive attention.

The presence of pathogenic microorganisms, antibiotic residues, and various chemical residues associated with cleaning or sanitation, represent the main fears of consumers and milk processors.

I.2. Physical and chemical composition of cow's milk [1]

From a physicochemical point of view, milk is a very complex product. A thorough knowledge of its structure is essential for understanding the transformations that take place in it and its derivatives during the various industrial treatments.

I.2.1. Milk composition [1]

Milk is a colloidal system made up of an aqueous solution of lactose, saline and several other elements in a dissolved state, in which there are proteins in the state of suspension and fat in the state of emulsion. The total dry extract of milk is on average 13.1% and the defatted dry extract (without fat) is 9.2%. The general composition of cow's milk is presented in Table I.2, the data of which are quantitative approximations, which vary depending on a multiplicity of factors: animal breeds, diet and state of health of the animal, lactation period, as well than during milking. However, the exact composition of a milk sample can only be obtained by analysis.

Component	**Average composition**
Water	86.9%
Fat	3.9%
Proteins and non-protein nitrogen compounds	3.2%
Carbohydrates	5.1%
Saline matter	0.9%
Minor constituent	
Enzymes, vitamins, Pigments (carotenes, xantophylls, riboflavin) Cells, various (epithelial cells, leukocytes, bacteria, yeasts, molds)	
Miscellaneous elements (carbon dioxide, oxygen, nitrogen and other gases) **Foreign matter**	

Table I.2. General composition of cow's milk

I.2.2. Physical and chemical properties of cow's milk [1]

Knowledge of the physicochemical properties of milk is of undoubted importance because it makes it possible to better assess the quality of the raw material and to plan the appropriate treatments and technological operations.

I.2.2.1. Milk acidity [1]

The pH (active acidity) of normal milk ranges from 6.2 to 6.8, but the majority of milks have a pH between 6.4 and 6.6. Colostrum is more acidic than normal milk, while milk at the end of lactation and that from sick cows generally has a higher pH, approaching the pH of blood.

All constituents capable of combining with basic ions contribute to the acidity of milk. It is the balance between the basic constituents (sodium, potassium, magnesium, calcium and hydrogen) and the acid constituents (phosphates, citrates, chlorides, carbonates, hydroxyls and proteins) of milk which determines its acidity. These two groups of constituents can exist in any combination. It should also be recognized that these combinations vary in degree of ionization, in dissociation constant and in solubility product. It should also be noted that the degree of dissociation increases with neutralization or pH and that calcium salts are less dissociated than sodium or potassium salts. It is for this reason that in milk, especially in an acidic environment, there is a predominance of calcium salts which tend to combine with proteins.

The acidity of the milk expressed as a percentage of lactic acid can vary from 0.10 to 0.30%. Most of the milks have an acidity of 0.14-0.17%. The natural constituents of milk which contribute to acidity are phosphates (0.09%), caseins (0.05-0.08%), other proteins (0.01%), citrates (0.01 %) and carbon dioxide (0.01%).

The acidity of milk can also be expressed in "Dornic degree". Fresh milk can have an acidity between 16 and 18° Dornic (with 1°D = 0.1 g of lactic acid per liter).

In dairy technology, we are particularly interested in changes in acidity during processing. Indeed, these changes can influence the stability of the constituents of milk.

Heating milk causes the loss of carbon dioxide, can break down lactose into various organic acids or cause blockage of amino groups in proteins, causing acidity to increase. Likewise, at high temperatures, tricalcium phosphate can precipitate and cause an increase in acidity triggered by the dissociation of phosphate radicals.

The development of lactic acid bacteria in milk transforms lactose mainly into lactic acid. It is this new acidity that is referred to as developed acidity and which leads to the destabilization of proteins. Depending on the use of milk, one can develop its acidity.

I.2.2.2. Freezing point [1]

The freezing point is the temperature of the transition from a liquid state to a solid state. It is one of the most stable constants in milk. This constancy results from the fact that the osmotic pressure of the milk is kept in equilibrium with that of the blood. The lowering of the freezing point is directly related to the solute concentration of a solution. It is therefore a measure of the number of molecules or ions in solution in the aqueous phase of milk.

The freezing point of milk can vary from -0.52 to -0.56°C; any variation greater than -0.52°C being a wetting index. It allows the detection of milk wetting from 3%. Lowering the freezing point can also be caused by the breakdown of lactose into several smaller molecules. It can also be used to assess the degree of hydration of proteins.

I.2.2.3. Boiling point [1]

At normal atmospheric pressure, the boiling point of water is 100°C and that of milk is 100.5°C. As with the freezing point, it is a function of the number of particles in solution and therefore increases with the concentration of milk and decreases with pressure. This phenomenon is applied in milk concentration processes.

I.2.2.4. Milk density [1]

The weight of a substance per unit volume is the density; while density is the ratio of density to that of water. Since the density of any substance varies with temperature, it is important to specify the latter when reporting the results. In practice, the density of water at 4°C is 1000 g /liter and therefore, at this temperature, the density and density of water are the same.

The density of milk at 15°C is on average 1.032 (1.028-1.035). It is the result of the density of each of the constituents of milk. For whole milk, the density should be measured at 30°C so that the fat is in a liquid state, otherwise, in the solid state; the fat has a higher and varying density.

I.3. Nutritional benefits of cow's milk [19] [33] [34]

I.3.1. Milk has a positive effect on health

Milk has its place in a balanced diet thanks to its nutritional richness. Globally, there is a scientific consensus on the usefulness of consuming milk at all ages. Many recent scientific studies show the positive effects of consuming milk and dairy products on human health.

However, in a diet, it's all about balance: it is important to respect the amounts to consume recommended in the food pyramid.

I.3.2. Nutritional wealth

Milk and dairy products are very rich in nutrition. They contain essential nutrients for humans. The composition of milk varies depending on the animal species, but all the nutrients are always present. For example, sheep's milk is particularly rich in fat and protein. Therefore, it is not used as fluid milk; it is almost exclusively processed into cheese and yogurt.

Average composition of 1liter of cow's milk:

- Water: 872 g
- Lactose: 48 g
- Fats: 37 g
- Proteins: 34 g
- Minerals : 9 g
- Vitamins : 1 g

I.3.3. Water

Milk contains 82 to 89% water depending on the species. Despite this, dieticians do not consider it as a drink but as a liquid food because of its richness in nutrients. This means that it should be consumed in sufficient quantity to cover the individual's needs, but not in excess.

I.3.4. Lactose

Most of the sugars in milk are lactose. This is digested in the intestine by lactase. Poor digestion of milk is often linked to not having enough lactase in the gut. It is lactose intolerance which affects between 10 and 20% of the Belgian population, for example, to varying degrees. Lactase is the enzyme in the digestive system of mammals that hydrolyses (dissociates) lactose into glucose and galactose, which are sugars that can be absorbed by the intestine.

I.3.5. Fatty substances

Milk fat is characterized by a very wide variety of fatty acids (over 400), 40% of which are short-chain unsaturated fatty acids. Milk fat also contains fat-soluble vitamins: vitamins A, D, E and K. Fat is found in milk in the form of fat globules surrounded by a membrane, certain constituents of which have positive effects on the body human health.

I.3.6. Proteins

Dairy proteins have a very good nutritional value thanks to their composition in essential amino acids. Amino acids are molecules that go into the composition of proteins. Essential amino acids are not synthesized by our bodies and must be obtained through food.

I.3.7. Minerals

Milk contains 22 essential minerals in the human diet. Milk and dairy products are best known for their high calcium content (1.25 grams per liter of milk) which helps prevent osteoporosis: half a liter of milk covers 75% of the daily needs in calcium from an adult. Milk is also a source of essential trace elements which can pose problems of insufficient human nutrition: zinc, iron, copper, iodine and selenium. The interest of milk minerals is their good bioavailability for the body. The bioavailability of a nutrient indicates the efficiency of the process of absorbing that nutrient through the intestinal wall into the bloodstream. Example: Calcium bioavailability refers to the amount of dietary calcium that can potentially be absorbed and the incorporation of this absorbed calcium into the bones.

I.3.8. Vitamins

Milk is an interesting source of vitamins for the whole population. Its different types of vitamins are:

- Fat soluble vitamins: there are vitamins A, D, E, K;
- Water-soluble vitamins: there are vitamins B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin), B9 (folic acid), B12 (cobalamin) and C (ascorbic acid).

Fat soluble vitamins are which are dissolved in the fat of milk. There are largely eliminated when the milk is skimmed.

The fat-soluble vitamins are dissolved in fat while the water-soluble vitamins are dissolved in the milk. It is for this reason that it is recommended to give whole milk to children, so that they get all the vitamins.

- **Vitamin A (or retinol):** It is present in milk fat, cheese, butter and cream. It is the main component of rhodopsin, an essential pigment of the retina, which must be constantly renewed, and which is necessary for vision in low light conditions. In addition, it participates in the regeneration of corneal cells.

It is also involved in the growth of bones, nails and hair, helps maintain the vitality of the skin and activates the immune system. The needs increase during breastfeeding, in people living in a polluted atmosphere or when taking the contraceptive pill.

- **Vitamin D**, vitamin pro-calcium (or calciferol): It plays an essential role in regulating the metabolism of calcium and phosphorus, of which it promotes intestinal absorption, and is therefore essential for optimal mineralization of bones and teeth.
- **Vitamin E** is a fat-soluble vitamin with several forms, but alpha-tocopherol is the only one used by the human body. Its main role is to act as an antioxidant, scavenging loose electrons—so-called "free radicals"—that can damage cells.
- **Vitamin K** refers to a group of fat-soluble compounds involved in coagulation, bone development, and cardiovascular health. Vitamin K deficiency can contribute to significant bleeding, poor bone development, osteoporosis, and increased risk of cardiovascular disease.

Water-soluble vitamins are vitamins which are soluble in milk water. There are:

- **Vitamins B1 and B2**: play a major role in the use of carbohydrates, lipids and proteins by our body, as well as in the functioning of our cells (enzymatic activity).
- **Vitamin B3 or Niacin** is a vitamin that's made and used by your body to turn food into energy. It helps keep your nervous system, digestive system and skin healthy. Niacin is often part of a daily multivitamin, but most people get enough niacin from the food they eat. Vitamin B3 is an important nutrient. It improves blood fat levels. Niacin may help to improve your blood fat levels, may reduce blood pressure, may help treat type 1 diabetes, boosts brain function, and improves skin health.
- **Vitamin B5** .In addition to playing a role in the breakdown of fats and carbohydrates for energy, vitamin B5 is critical to the manufacture of red blood cells, as well as sex and stress-related hormones produced in the adrenal glands, small glands that sit atop the kidneys.
- **Vitamin B6** helps maintain a normal amount of this amino acid in your blood. It is a stronger immune system. Vitamin B6 helps chemical reactions in the immune system, helping it work better. Eating foods rich in vitamin B6 will help your body guard against infection.
- **Vitamin B7** promotes appropriate function of the nervous system and is essential for liver metabolism as well. Biotin is commonly advised as a dietary supplement for

strengthening hair and nails, as well as in skin care. It is suggested that biotin aids cell growth and the maintenance of mucous membranes.

- **Vitamin B9**, which contributes to the formation of red blood cells and which is involved in the production and transformation of proteins. This vitamin is mainly found in blue cheeses (Roquefort) or with a bloomy rind (Camembert, Coulommiers, Brie, etc.).
- **Vitamin B12**, necessary for the formation of red blood cells. It also participates in the manufacture of proteins and is involved in the functioning of the nervous system. It is found almost exclusively in foods of animal origin.
- **Vitamin C,** also known as ascorbic acid, is necessary for the growth, development and repair of all body tissues. It's involved in many body functions, including formation of collagen, absorption of iron, the proper functioning of the immune system, wound healing, and the maintenance of cartilage, bones, and teeth.

The nutritional recommendations of the food pyramid recommend consuming 2 to 3 dairy products per day. These recommendations consider milk and dairy products to be a source of calcium easily absorbed by the body. They take into account the eating habits of people. They are also a function of the age and sex of the person.

On October 1, 2019, the Superior Health Council published the new nutritional recommendations. They recommend a consumption of 250 to 500 ml of milk or dairy products per day for an adult.

I.4. Milk microbiology [1]

Microbiology is closely linked to the dairy industry: it applies to all its sectors. Its principles, in fact, justify the hygienic mode of milk production, command several treatments and industrial processes during its transformation in the factory, and are the basis of the methods of preserving dairy products. The quality of milk and dairy products largely depends on it, so microbiological standards are taken into account in its official assessment.

The application of general principles of hygiene makes it possible to achieve the following three goals:

- The first, to prevent and prevent the transmission of pathogenic bacteria through milk and dairy products and in this way protect the health of consumers;
- The second, to prevent and restrict microbial growth in milk and dairy products and thus prevent their deterioration and the appearance of defects;

- The third, to promote and guide the development of useful bacteria in certain dairy products, such as fermented products (yogurt, kefir, Smetana, etc.).

I.4.1. Classification of microorganisms associated with milk [1]

It is possible to establish an identification key allowing recognizing the species which one meets most frequently in a product. The method of identification used is mainly based on the tests of Gram, catalase, oxidase, fermentation of sugars and sporulation, the methodology of which, in the case of the first four, can be found in any manual of general microbiology...

For its part, sporulation can be recognized by the survival of an organism subjected to heating at 85°C for 10 minutes.

Table I.3 presents the classification of some bacterial species associated with milk, obtained using those tests.

Gram stain	**Form**	**Action**	**Name**
Gram (+)	Cockles	Catalase (+)	*Staphylococcus, Micrococcus*
		Catalase (-)	*Streptococcus, Pediococcus, Leuconostoc*
	Sticks	Sporulated	Aerobic: *Bacillus* Anaerobes: *Clostridium*
		Non sporulated	*Lactobacillus*
Gram (-)	Sticks	Oxidase (+)	Saprophytic, often psychrotrophic: *Pseudomonas, Flavobacterium*
		Oxidase (-)	• *Enterobacteria: Escherichia, Enterobacter, Citrobacter, Klebsiella, Salmonella, Shigella...* • *Xanthomonas …*

Table I.3. Classification of some bacteria associated with milk

When we see the bacterial composition of raw cow's milk, it is a potentially dangerous food that must be processed and protected to assure its safety for humans.

Furthermore, *Brucella* can be found in milk and contaminate humans through the consumption of contaminated food (mainly milk and raw dairy products) and through contact with the skin (even apparently healthy) or mucous membranes (digestive, conjunctival and naso-pharyngeal) with infected animals and their products (mainly genital secretions, abortions and placentas but also infected organs, liver, spleen, udder in particular, as well as contaminated manure or wool) **[23].**

There are also indole bacteria which are bacteria that break down the protein tryptophan into indole.

Sulfhydrogen (or putrid) bacteria are bacteria that produce H_2S (hydrogen sulfide) from sulfur amino acids or mineral forms of sulfur (sulfate, sulfite, etc.). Sulfhydrogen bacteria are not necessarily sulfito-reducing.

Their interest in the Food Industry is that the indologenic and putrid flora can cause:

- Degradation of the food likely to lead to manufacturing accidents (putrefaction);
- Changes in the taste or smell of the food with H_2S in particular.

Foods rich in amino acids (dairy products and meat products) are the most sensitive to the action of this flora **[23].**

I.4.2. Factors in the evolution of the microbial flora [1] [7]

The multiplication and virulent potential of those microorganisms can be reinforced by several contamination parameters during milking:

- The cow itself, if it is sick, can excrete *Brucella, Mycobacterium* in the milk;
- The environment:
- Soil and plants almost all bring germs, in very large quantities.
- Natural, untreated water, from a source or from a river, also brings many germs, which often come from the soil. However, we can also find pollution germs, resulting from runoff: *Enterobacteria* (from animal feces), *Clostridium*, including certain pathogenic germs such as *Salmonella typhi*.
- Air and dust are mainly transport agents. They are essentially vectors of exogenous spores and bacteria.
- Humans are a very contaminating agent by:
- Their hands: the skin contains permanent germs, such as certain Staphylococci, and often pathogenic transition germs (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas*, etc.). The hands can carry *Enterobacteriaceae* of fecal origin.
- Their nasal or oral secretions may contain *Staphylococci.*
- Their dust-bearing clothes are therefore carriers of mold and spores.
- Equipment and instruments carry germs from contaminated water or other food in the event of poor cleaning and disinfection practices.
- Milking methods can be a disorganization of production by:
- Cross contamination;

- A loss of risk control.

Microorganisms are present in all foods. But microbial populations do not stay fixed. They evolve according to:

- Nutrients (sources of energy: carbohydrates above all, lipids possibly, sources of assimilable nitrogen, necessary for the production of proteins and genetic factors: amino acids, simple peptides, growth factors: vitamins, certain minerals).
- Water is both a carrier of germs and an essential element for life. It is fundamental for the multiplication of germs and the germination of spores.
- pH: The ranges used are for molds: pH between 1.5-2 and 11; optimum: 7; for yeasts: pH between 2.5 and 8.5; optimum: 6.5 and for bacteria: pH between 3.8 and 9; optimum: 7.
- Other factors: redox potential, temperature, etc.

To attract everyone's attention, we are therefore going to give all the probable poisonings due to the consumption of raw cow's milk, of course, but also to the consumption of any food product that can cause food poisoning; especially since certain strands of food (cereals, legumes, meat, etc.) can be found in the milk during milking and sale.

I.4.3. Food poisoning

I.4.3.1. The presence of a toxic chemical

I.4.3.1.1. Industrial chemical contaminants

These chemicals are not related to the presence of microorganisms; it can be due to fraud, criminal acts, pollution. These are toxic compounds and elements (Lead, Zinc, Cadmium, Mercury, Arsenic, Cyanides), food additives, vitamins and minerals, contaminants (lubricants, cleaning agents, disinfection agents, protection agents, paints, coolants, boiler water treatment agents, rat poison, insecticides), polybiphenyl chlorides (PCBs), agricultural products (pesticides, fertilizers), antibiotics, growth hormones, prohibited products (direct or indirect), etc. **[16] [22].**

I.4.3.1.2. Contaminants from packaging

These are the plasticizing compounds, vinyl chloride, labeling ink, coding, adhesives, lead, aluminum, tin, etc. **[16].**

I.4.3.1.3. Natural chemical compounds

These products can be produced by microorganisms which are not then directly responsible for the poisoning: histamine poisoning (histidine decarboxylation product, amino acid), poorly understood poisoning due to various metabolic products (allergens, pyrrolytic alkaloids, phytohemagglutins ...) **[16] [22].**

I.4.3.2. The presence of microorganisms and or their toxins

I.4.3.2.1. Contamination

It is from various origins:

- It can be original: sick animal or healthy carrier of germs, vegetables soiled by manure or waste water or often during the handling of food by a sick person or a healthy carrier of germs;
- It can also be done through insects, flies, rodents, domestic animals;
- It can also come from appliances used for food preparation (mincer, grinder, etc.) **[22].**

I.4.3.2.2. The multiplication of contaminating bacteria

During the food poisoning phenomenon, one must take into account the quantity, quality (virulence) of the germs and the resistance of the host organism. The number of germs ingested according to age can be a danger.

This last factor involves two concepts: intoxinations and toxic-infections **[22].**

- **Intoxinations:**

Poisoning is distinguished by the following characters:

- The pathological manifestations of poisoning are those of the toxin produced by a microorganism;

- Microorganisms do not develop (or very little) in the patient;

- The toxin is protein or peptide in nature. A toxin is a molecule exhibiting both a toxic and antigenic character **[22] [29].**

- **Toxic-infections:**

Living microorganisms, present in food, by their multiplication in the individual first (virulence) and possibly by the production of protein toxins or glucidolipidoprotein, cause pathological manifestations. As indicated above, we will only consider the study of intoxinations and toxic-infections because they represent at least 70% of food poisoning **[30].**

I.4.3.3. Intoxinations

I.4.3.3.1. *Clostridium botulinum*

I.4.3.3.1.1. Bacteria

Clostridium botulinum is a Gram +, sporulated, strict anaerobic, protein toxin-producing bacillus.

Clostridium botulinum is found in soils (soil bacteria), in the intestines of animals such as pigs and possibly humans. It is therefore saprophytic or commensal. However, the land-based origin may be related to fecal contamination.

The spore, a particular form of the bacterium, is particularly resistant to temperature like that of another *Clostridium* and *Bacillus* **[22] [30].**

I.4.3.3.1.2. Conditions of toxinogenesis [22] [29]

For toxinogenesis to occur, it is essential for the bacteria to multiply in the food, which presupposes at least three conditions:

- Anaerobiosis;

- PH of the medium greater than 4.5 (in general);

- NaCl concentration of the medium less than 10g/l.

And in some cases:

- Sterilization scale (100°C, 10 min) not respected;

- Refrigeration temperature above 4.5°C;

- Growth temperature above 10°C.

I.4.3.3.1.3. Foods involved

Family preserves of fruits and vegetables, raw ham, dried and salted meat and fish, smoked fish, unpasteurized honey, etc.

I.4.3.3.I.4. Symptoms

There are nausea and vomiting, speech difficulties, double vision (diplopia), fixed and dilated pupils, paralysis of the muscles, etc. **[22].**

The first manifestations usually do not appear until three to six days after consumption, but they can start either within a few hours and progress very quickly to death or only after a week and last over a fortnight. When the patient recovers (which often requires very rapid specific treatment), he does not really "recover" until after more than six months **[11].**

The effects of botulinum toxins are sensitive at extremely low doses: the minimum lethal dose is around 30pg/kg. It takes 10 times more tetanus toxin and 40,106 times more cyanide to achieve such toxicity. Botulinum toxins are therefore the most effective poisons known **[11] [22] [29].**

According to Oteng-Gyang **[29],** it has been estimated, with tests and calculations based on mice, that a dose of 0.2 g would suffice to kill a human weighing 100 kg.

I.4.3.3.1.5. Prevention

The prevention that follows is therefore to refrigerate food products, especially those made from meat and fish, at a temperature below 4.5°C to prevent the multiplication of germs. Products preserved at home should also be heated for at least 10 minutes at 100°C before consumption **[29].**

When canning low-acid foods, you should also pay attention to the duration of the canning to eliminate any endospores formed by bacteria.

To ensure destruction of all endospores, heat 6 hours at 100°C, 2 hours at 105°C, 12 minutes at 115°C or 4 minutes at 120°C **[30].**

I.4.3.3.2. Enterotoxic staphylococci

I.4.3.3.2.1) Bacteria

The enterotoxin-producing Staphylococci are certain *Staphylococcus aureus*. They are spherical bacteria, facultative aero-anaerobes, catalase +, which can give colonies pigmented in golden yellow.

Their habitat is very variable, often the mucous membranes of humans and animals, and they can be the source of infections, especially skin infections (boils, whitlow, etc.). The pathogenic strains are enterotoxinogenic or not. Food poisoning from *Staphylococcus aureus* is due only to enterotoxin and not to virulence (a general property in *S. aureus*) **[22] [29].**

I.4.3.3.2.2. Toxinogenesis condition

Producing Staphylococci secrete the toxin when they multiply. It is enough, therefore, that the conditions are favorable for the cultivation of Staphylococci for the enterotoxin to be found in the food. These conditions are the availability of nutrients and a temperature between approximately 10 and 50°C **[22].**

I.4.3.3.2.3. Foods and staphylococcal toxins

The food causing poisoning contains large amounts of the toxin. It is in the food that the Staphylococci produced the toxin. They are therefore present in large quantities if heating the food has not killed them.

The foods involved are very varied, especially pastries and creams, and often consumed 12 to 24 hours after their manufacture, which gives Staphylococci time to multiply by producing their enterotoxin.

The origin of Staphylococci is most often a skin infection of kitchen staff **[22].**

The symptoms are headache, nausea accompanied by vomiting, diarrhea; the first symptoms last from 1 to 6 hours **[29].**

I.4.3.3.2.4. Prevention

- Cook food properly, i.e. at temperatures of at least 70°C;

- Refrigerate perishable foods at temperatures below 6°C or keep them at a temperature above 47°C;

- Do not allow food to be handled by people with skin lesions or carriers of *Staphylococcus aureus* **[30].**

I.4.3.3.3. Mycotoxins

I.4.3.3.3.1. Definitions

Molds are filamentous fungi that are common contaminants in food products. They are saprophytes with great degradation power. Some species are widely used in industry (cheese, antibiotics). Others are toxigenic (producing mycotoxins).

Mycotoxins are therefore organic substances synthesized by microscopic fungi (molds) and exhibiting toxicity for humans or animals **[2].**

I.4.3.3.3.2. Some mycotoxins produced by molds on food and the syndromes they cause [29]

Mycotoxin	Mold	Food	Syndrome
Aflatoxin (B1, G1, B2, G2, M1, M2, P1)	*Aspergillus flavus*, *Aspergillus parasiticus*	Peanuts, cereals	Hepatic syndrome, hepatocarcinoma
Ergotamine	*Claviceps purpurea*	Rye	Ergotism (Fire of Saint Antony), gangrene
Aspergillosis toxin	*Aspergillus fumigatus*	Cassava, artichokes	Hemolytic effects
Ochratoxin (A)	*Aspergillus ochraceus*, *Penicillium viricatum*	Maize, barley	Damage to the liver and kidney
Zearalenone F2, F3	*Fusarium graminearum*	Maize, hay, food	Estrogenic effects
Citrinin	*Penicillium citrium*	Barley, rye, oats	Renal syndrome
Patulin	*Aspergillus clavatus*, *Penicillium expansum*, *Pénicillium patulum*	Wheat germs, potato, soil, compost, fruit, wheat, straw	Neurotropic effects, pellagra, epithelioma
Penicilloic acid	*Penicillium cyclopium*, *Penicillium viridicatum*	Cereals	Epithelioma, pellagra, coma
Cladosporic acid	*Cladosporium*	Cereals, millet	Aleukemic, food poisoning
T2 toxin	*Fusarium tricinctum*	Maize	Hemorrhagic syndrome
Sterigmatocystin	*Aspergillus versicolor*	Wheat	Hepatocarcinoma
Cytochalasin	*Cramped phoma*	Potato	Teratogenic effects
Luteoskyrin, Cyclochlorotin Island toxin	*Penicillium islandicum*	Rice	Cirrhosis, tumors, severe liver damage, atrophic liver

Table I.4. Mycotoxins and syndromes caused

I.4.3.3.3.3. Factors promoting toxinogenesis

The development of toxigenic molds is a necessary condition, but it is not a sufficient condition. For a genus known to be toxinogenic, the aptitude for toxinogenesis is a genetic

factor (subject to mutation and selection) but the expression of this aptitude often depends mainly on the main characteristics of the environment: humidity, temperature, nature of the food, pH, composition of the gas phase, nature of the substrate, etc. **[29] [30].**

I.4.3.3.3.4. Symptoms of mycotoxinosis

Diarrhea and vomiting occur about 24 hours after eating the mushrooms. Then, hepatic, renal and neurological manifestations appear **[26].**

I.4.3.3.3.5. Prevention

- Do not exceed during storage a temperature of 30-35°C and relative humidity must not be high (must be less than 80%);

Molds are thermostable and are degraded at temperatures above 150°C during technological treatments **[30].**

According to Scharma **[29],** onion extracts have antifungal activities. As for Buchanan and Fletcher **[29],** they believe that caffeine is able to inhibit the growth of molds.

I.4.3.4. Food poisoning (Toxic-infection)

I.4.3.4.1. Salmonellosis

I.4.3.4.1.1. Definition of *Salmonella*

Salmonella are *Enterobacteriaceae* belonging to a single species: *Salmonella enterica*. They are Gram (-), rod-shaped, peritrichous, facultative aero-anaerobic bacteria. They ferment glucose to produce gas and acid. But they do not ferment sucrose or lactose. *Salmonella* are urease (-), produce hydrogen sulfide and can decarboxylate some amino acids. There are over 3,500 different serovars known to date **[22].**

I.4.3.4.1.2. Habitat and pathogenicity of *Salmonella*

They are generally pathogenic for humans and animals. The pathogenicity in humans must allow us to distinguish two categories of *Salmonella*:

- Those giving specifically human salmonellosis: typhoid and paratyphoid fevers, serious long-term diseases (because these *Salmonella* are disseminating);

- And those which are often of food origin **[29].**

Typhoid fever manifests as a very high temperature with ulceration of the small intestine. It is possible that a perforation of the intestine is involved. Death usually occurs from fever or from perforation of the intestine leading to peritonitis **[22].**

The foods involved are meats, eggs, creams, dairy products, water, shellfish, etc.

Symptoms are diarrhea, nausea, abdominal cramps, vomiting, fever of 38-39°C; the first signs appear 6 to 48 hours after consuming the contaminated food **[22].**

I.4.3.4.1.3. Prevention

Since Salmonella are heat sensitive, they are destroyed by exposure to 10 minutes at 70-80°C.

It is therefore always preferable to:

- Maintain at 70°C at least, creams and egg-based sauces which do not tolerate boiling well; -
- Ensure that the poultry are sufficiently cooked at a temperature above 70°C;

- Systematically and carefully clean his hands, cutting boards and utensils after preparing poultry;

- Store cooked food in the refrigerator;

- Refrigerate food appropriately;

- Protect food from contamination by rodents and domestic animals;

- Have personal cleanliness **[22].**

I.4.3.4.2. *Clostridium perfringens* (type A) toxic-infections

Some *Clostridium perfringens* (type A) are capable of producing a toxin. In a food cooked in broth, destruction of the spores does not take place **[22].**

Its Source of infection is fecal matter **[13].**

The foods implicated are raw, untreated meats, meats in sauce.

The symptoms are intestinal cramps, nausea, diarrhea; the first appear 8 to 22 hours after consuming the contaminated food.

Prevention is achieved by cooking food at temperatures above 65°C, by refrigerating the food **[22] [29] [30].**

I.4.3.5. Other toxic-infections

I.4.3.5.1. Bacterial toxic infections

- ***Shigella*** (and in particular *Shigella sonnei*)

The sources of infection are feces and the implicated foods are meats, eggs, creams, milk, produce.

Symptoms manifest as bloody diarrhea and abdominal cramps; the first symptoms appearing 15 to 7 days after consumption of contaminated food **[22] [29] [30].**

Prevention is done by refrigerating food, the strict hygiene of food handlers **[22].**

- ***Yersinia enterocolitica*** recently suspected, bacteria multiplying well at 4°C. Yersiniosis is characterized by "pseudo-appendicitis" abdominal pain with attacks of arthritis, diarrhea and ulcerations of the intestinal mucous membranes; vomiting and nausea occur inconsistently.

The foods involved are: pork and other animals and the sources of infection are feces. The first symptoms last from 24 to 36 hours.

As prevention, food must be sufficiently cooked, elementary hygiene rules associated with decontamination treatments, milk pasteurization, and treatment of raw meats with lactic acid **[22].**

- ***Bacillus cereus*** incriminated for very contaminated mashed potatoes, pastry creams or rice. These bacteria cause more diarrhea and colic than vomiting 8 to 24 hours after consuming contaminated food. Digestive disorders disappear quickly. It can be intoxination as well as toxic-infection.

The sources of infection are: soil, air, animals, and healthy carriers.

Prevention is achieved by cooking food sufficiently, by respecting elementary hygiene rules **[22].**

- Enterotoxic or enteroinvasive ***Escherichia coli*****:** Fecal in origin, they are found in food contaminated by the feces of infected animals and healthy humans. 6 to 36 hours, after contamination of contaminated food, diarrhea, nausea, abdominal cramps, vomiting appear.

Prevention is achieved by sufficient cooking of food (over 70°C), refrigeration, and strict hygiene of food handlers **[22] [30].**

- ***Campylobacter jejuni***, of fecal origin, is found in poultry, milk, and water. It is responsible for gastroenteritis of the same severity as salmonellosis and shigellosis with a higher frequency in children under three years old.

Campylobacteriosis are characterized by diarrhea, abdominal pain with sometimes fever and vomiting 2 to 7 hours after consuming the offending foods. Sometimes complications up to septicemia, meningitis, etc. are noticed.

Contamination occurs through ingestion of contaminated food or water. This germ can survive two to three weeks in cow's milk or feed water stored at 4°C. The most frequently implicated foods are unpasteurized milk, poultry meat and feed water. The infectious dose varies from 500 to 10^6 cells depending on the subject **[11] [22].**

Prevention is done by irradiation of food, strict control over the herds.

- ***Listeria monocytogenes***, Gram + bacteria, catalase +, ubiquitous and therefore isolated from poultry, meat, water and milk.

From these different food products, *Listeria monocytogenes* can reach the human body where it can cause infections (septicemia and meningitis) especially in susceptible individuals. It can also cause miscarriages, premature births. Because of its temperature sensitivity and its sensitivity to disinfecting agents, *L. monocytogenes* is rarely isolated from industrially processed foods.

Prevention is done by pasteurizing the offending foods **[22].**

- ***Vibrio parahaemolyticus***, a halophilic vibrio found in seafood. It can also obviously contaminate other foods by direct contact or by the fingers which successively handle them. It manifests as *Bacillus cereus*, but after 12-24 hours.

Prevention is achieved through proper cooking of food, refrigeration, strict hygiene of food handlers **[30].**

- ***Vibrio cholerae*** causing cholera. It is an often-fatal disease transmitted through food. It is characterized by acute diarrhea with excruciating pain and paralysis after 3 to 5 days.

Death is caused by dehydration and can easily be avoided if the patient is given a drink of lightly salted and sweetened water (compensation for mineral losses). Prevention must also be carried out by cooking food sufficiently, by respecting elementary hygiene rules **[29].**

- ***Brucella (abortus, melitensis, suis)***: Its sources of infection are: sheep, cattle, goats, pigs. Raw milk and butcher's meat are the foods implicated.

Symptoms are manifested by fever (40°C), ill-defined discomfort, lack of appetite, abortions for the woman and the animals.

Prevention is done by pasteurization of milk, surveillance and vaccination of animals, sufficient cooking of meat **[30].**

- ***Mycobacterium bovis*** **[3].**

Bovine tuberculosis is a contagious, debilitating disease of humans and animals. It is caused by the bacillus *Mycobacterium bovis* (*M. bovis*) belonging to the *Mycobacterium* complex, which also includes *M. tuberculosis* and *M. avium*. The lymph nodes are the primary site of infection, but other organs such as the lungs are also affected when the disease is at an

advanced stage. The clinical signs of the disease are weakness, loss of appetite, weight loss and fever.

Bovine tuberculosis usually hosts cattle, but it can be transmitted to humans as well as to other animals such as pigs, bison and deer (deer and elk). The bacillus does not survive exposure to heat, sun or drought, and it does not replicate outside its hosts. People at greatest risk of contracting *M. bovis* bacillus are those who have direct and prolonged contact with infected animals, such as farmers, agricultural workers and veterinarians. The most common way to get the disease is by inhaling aerosols released from the breath and cough products of a sick animal. The risk of contamination is particularly high in enclosed places such as livestock buildings. Other modes of contamination are ingesting unpasteurized milk from an infected cow and sharing the same sources of water or feed.

I.4.3.5.2. Viral infections

Viral infections can be real food poisoning. They manifest as diarrhea that lasts 24 to 48 hours. This diarrhea is associated with abdominal pain and vomiting, often accompanied by fever, anorexia and myalgia. The incubation period is short, ranging from 15 hours to 3 days **[22].**

This is the case, for example, with viral hepatitis A and E transmitted by contaminated shellfish. Other viruses can be involved: *Rotaviruses, Norwalk virus, Adenoviruses, Coronaviruses, Caliciviruses, polio virus* transmitted by contaminated water etc**. [22] [31].**

I.4.3.5.3. Parasitic poisoning [22] [30]

The disorders are sometimes similar to bacterial poisoning. Their presence in food is often the result of fecal contamination. Among these parasites, let us note:

I.4.3.5.3.1. Nematodes (roundworms)

- ***Trichinella spiralis*** or Trichina is an intestinal parasite, the larvae of which will lodge in the muscles of the parasitized individual and become encysted there. Eating contaminated meat triggers parasitosis. The symptoms are characterized by edema in the face. We can observe diarrhea, fever.
- ***Ascaris lumbricoïdes*** is an intestinal worm whose eggs are brought by food soiled with soil in which human feces have been deposited. After passage through the lungs, the larvae give birth to adults living in the intestine; the stool will contain the eggs. It is a parasitosis manifested by abdominal pain, nausea, vomiting, and anorexia.

Other nematodes can have a food origin: Oxyure (*Enterobius vermicularis*) which causes anal pruritus in addition to the signs of Ascaris.

Whipworm (*Trichuris trichiura*) is manifested by acute dysentery, rectal prolapse, and anemia.

I.4.3.5.3.2. Flatworms (Tapeworms)

- **Pork Tænia** (***Tænia solium***) and **beef Tænia** (*Tænias saginata*) are transmitted by cysterical larvae contained in the muscles of pork or beef. The larvae transform into the large size worms that live in the host's intestine. This is the tapeworm for Beef Taenia. The stool contains the eggs which will contaminate the intermediate animals, beef and pork.

Taeniasis is manifested by anorexia, nausea, diarrhea, epigastric cramps.

- **The Greater Fluke** (***Fasciola hepatica***) is transmitted by encysted larvae on watercress. These larvae turn into tapeworms that live in the bile ducts. The eggs are emitted in the stool and contaminate an aquatic gastropod which will emit larvae attaching to the watercress. This parasitosis is particularly serious in sheep.

There is abdominal pain, diarrhea, hepatic cholic attacks, asthenia and also jaundice.

I.4.3.5.3.3. Protozoa

- ***Toxoplasma*** **(*Toxoplasma gondii***) forms cysts located in the muscles in parasitized individuals (mammals, birds, invertebrates, etc.). Consumed by a predator, these cysts will give back to toxoplasma which will encyst again. In cats, the toxoplasma can undergo an intra-intestinal sexual cycle giving rise to oocysts which, released in the feces, can infect other animals, in particular herbivores. An individual can therefore be infected either by oocytes from cat feces, or by eating meat containing cysts or undercooked. Freezing does not kill cysts.

There are two clinical forms: toxoplasmosis acquired after birth and that which is congenital.

That which is acquired, is benign and manifests itself in the form of fever, and asthenia.

On the other hand, that which is congenital manifests itself by abortion, visceral damage, and hydrocephalus.

- **Dysenteric amoeba** (***Entamoeba histolytica***) is transmitted by cysts from the feces of infected individuals and often triggers dysentery. This dysentery is characterized by

frequent mucus-bloody stools (5 to 17 time /day). ***Giardia intestinalis*** works the same way.

I.4.3.5.3.4. Prevention of parasitosis

For most of these parasitic diseases, cooking the food and sometimes freezing it destroys the parasite. Eliminating intermediate hosts is an effective solution for the Moat. The fight against fecal peril limits dissemination.

I.4.4. Prevention of foodborne illness [22]

I.4.4.1. Sanitation

Sanitize by destroying microorganisms including spores and toxins: respecting the cooking time and temperature.

I.4.4.2. Prevention and remediation

The necessary conditions for the applications have: control of the whole chain and training, awareness, education of staff in order to motivate and involve them.

I.4.4.3. Prevention

1) Avoid the contributions of microorganisms:

- By ensuring the maintenance of premises and equipment;
- By using only healthy products and protecting them;
- By closely monitoring the health and hygiene of staff;
- By avoiding contact of healthy foodstuffs with soiled areas.

2) Limit multiplication:

- By bringing consumption closer to the preparation for foodstuffs with limited storage;
- By ensuring that the danger zone (from 10 to 65°C) is crossed quickly in less than 2 hours;
- By respecting the heating chain (temperature above 65°C for consumption cooking);
- By respecting the cold chain (between 0 and 3°C for refrigerated products and below – 18°C for frozen products).

I.4.5. Microorganisms sought in foods [23]

I.4.5.1. Microorganisms that affect the marketable quality of food

They are microorganisms of all kinds and whose presence in the product does not present a specific danger for the consumer but rather for the product. These are the aerobic mesophilic

germs which develop on ordinary medium, some yeasts and molds are therefore part of this group of germs.

I.4.5.2. Marker microorganisms

There are two types of marker microorganisms: index and indicator.

I.4.5.2.1. Index microorganisms

They indicate or suppose the possible presence of pathogenic microorganisms of the same ecological origin. Those are:

- *E. coli* whose presence in food assumes that of *Salmonella, Shigella, Vibrio* or hepatitis A virus in this food analyzed because it is from the same habitat.
- Fecal or thermotolerant coliforms with the same meaning as *E. coli*.
- Sulfite-reducing anaerobes: their presence in food reflects a risk of *Clostridium botulinum* infection (witness to old fecal contamination).

Most indexes are therefore enteric microorganisms that cannot live very long outside this habitat. This last criterion excludes enteric germs such as enterococci and *Clostridium perfringens* which sometimes resist for a very long time outside the intestine; their immediate origin is therefore not necessarily the intestine and therefore not index.

I.4.5.2.2. Indicator microorganisms

These are microorganisms whose presence in a food or on a facility indicates an insufficiency of a given operation according to the indicator.

- Group D streptococci (fecal *Streptococci*) indicate insufficient cleaning or disinfection with chemical agents.
- Total coliforms and *Enterobacteriaceae* in general present in food, reflect insufficient heat treatment of the product analyzed.
- *Bacillaceae* and *Clostridium* spores are witnesses of recontamination after heat treatments.
- Yeasts and molds in a dry product indicate recontamination of the product after treatment.
- The presence of indologenic and sulfhydrogenic bacteria reflects a lack of food preservation technology.

By decreasing fecal specificity, we have *E. coli*, fecal coliforms, fecal streptococci and sulfite-reducing clostridium spores. On the other hand, by increasing external resistance, *Clostridium* sulfite-reducing spores come first.

I.4.5.3. Pathogenic microorganisms (research and enumeration)

They are toxigenic or invasive microorganisms, causing more or less serious disorders in the body that ingests them. The main bacteria responsible for poisoning are presented in the tables below with the characteristics of the toxins.

- **Bacteria responsible for poisoning and their toxins: the toxin is preformed in the food.**

Bacteria	Toxin	Activity
Brucella, Staphylococcus aureus	Enterotoxin	Neurotoxic
Bacillus cereus	Emetic, enterotoxin	Emetic, enterotoxic
Clostridium botulinum	Toxins A, B, C_1, C_2, D, E, F	Neurotoxic except C_2

Table I.5. Bacteria responsible for poisoning and their toxins: the toxin is preformed in the food

- **Bacteria responsible for intestinal infections and their toxins: the toxin is preformed at the site of infection**.

Bacteria	Toxins	Entero-toxic	Cyto-tonic	Cyto-toxic	Other
E.coli					
EPEC	Shiga Like Toxin (SLT)	+		+	
EHEC	Shiga Like Toxin (SLT)	+		+	
ETEC	Heat labile toxin (LT)	+	+		
EIEC	Thermostable toxin (ST)	+		+	
Shigella sp	Shiga Toxin	+		+	Neuro-
Salmonella sp	Enterotoxin	+		+	toxic
Yersinia enterocolitica	Toxin ST	+	+		
Campylobacter jejuni	Toxin (CJT)	+		+	
Vibrio cholerae	Cholera Toxin (TC)	+			
Vibrio parahaemolyticus	Thermostable Hemolysin	+		+	Lethal cardio-
Clostridium perfringens type A	Enterotoxin	+			toxic
type C	Toxin ß	+			
	Toxin γ			+	
	Toxin θ			+	

Table I.6. Bacteria responsible for intestinal infections and their toxins: the toxin is preformed at the site of infection

- **Example of summary about *E. coli***

Figure I.1. Example of E. coli research pathways

II. SECOND PART: METHODOLOGY (MATERIALS AND METHODS)

II.1. Context of the analyses

The nutritional quality and microbiological quality of raw cow's milk will be assessed by nutritional analysis and counting of microorganisms.

II.1.1. Nutritional analysis

The organoleptic qualities: appearance and texture, smell, color, density, pH, Dornic acidity, and viscosity, etc., will be done on site or upon our arrival at the laboratory, to accompany the nutritional quality.

The analysis or dosage of nutrients in the laboratory will particularly concern water, carbohydrates (lactose), lipids, proteins, minerals, vitamins, by volumetry or colorimetry, due to the lack of more sophisticated and efficient devices: HPLC, PCR, GC, etc.

II.1.2. Enumeration of microorganisms in foodstuffs

In food microbiology, three groups (types) of microorganisms are distinguished which are:

- Microorganisms affecting marketable quality: mesophilic aerobic germs (to be counted), some yeasts and molds (fungal flora);
- Index or indicator microorganisms: Coliforms, Sulphite-reducing anaerobes, *Streptococci*, *Enterobacteria,* Indologenic and putrid bacteria (to be counted with coliforms);

Those two groups are called general hygiene test microorganisms.

- Potentially pathogenic microorganisms: *Salmonella*, *Staphylococci*, *Vibrio*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Brucella* (to be examinated under the microscope with *Mycobacteria*), etc.

Our study will consist in the exposition of our working methodology, in which we will:

- Give the methodology used relating to the identification, the sampling periods and frequencies, the provisional work schedule, the selection of the sampling points and conditions and sampling method, the campaign sheet;
- Identify methods and materials and perform nutritional analyses;
- Give the criteria, the calculation of the dilution intervals, highlight the analysis scheme, identify the equipment, materials, diluents, culture media and reagents and count and search for microorganisms in the laboratory.

II.2. Methodology used

II.2.1. Identification, sampling periods and frequencies

The sampling campaigns will be carried out at the Bagnon market in Yopougon-Abidjan (Côte d'Ivoire) at the various points of sale. We situate below the geographical location of Yopougon among the ten municipalities of Abidjan.

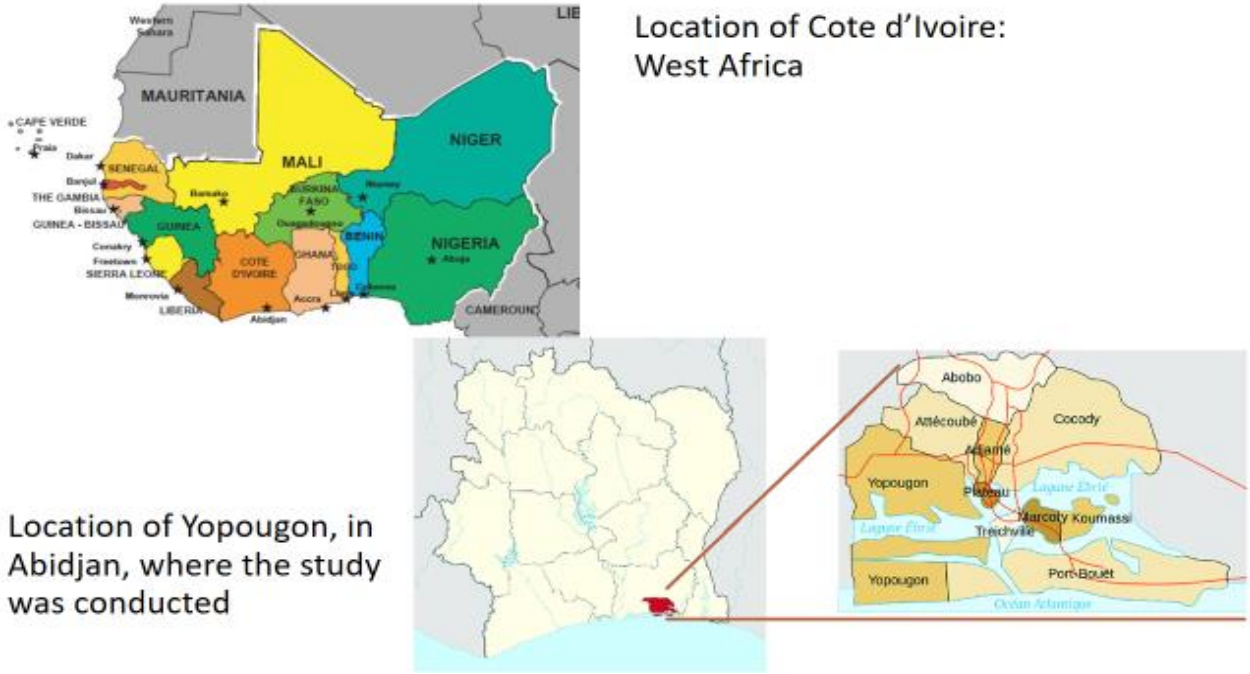

Figure II.1. Yopougon, among the ten (10) municipalities of Abidjan

In tropical climates, proliferations of microorganisms can occur all year round **[17].** That is why we are going to carry out four **(4**) sampling campaigns per year, including one per quarter **(T)),** taking into account the different seasons in Côte d'Ivoire. They will begin the first week of March 2022 and end in the first week of December 2022. The samples will be taken as follows (unless there is a major problem):

- The first campaign will take place around mid-March 2022, most often corresponding to the end of the long dry season;
- The second will be done around mid-June 2022, most often corresponding to the long rainy season;
- The third is carried out in the second week of September 2022, most often corresponding to the short dry season and the hot period (temperature ≥ 30°C) **[17]**;
- The fourth will take place between the end of November and the end of December 2022, most often corresponding to the start of the major dry season.

II.2.2. Provisional work schedule

Activities	Year 2022			
	T1	**T2**	**T3**	**T4**
1st sampling and laboratory analysis				
2nd sampling and laboratory analysis				
3rd sampling and laboratory analysis				
4th sampling and laboratory analysis				
Final drafting				

Table II.1. Provisional work schedule

II.2.3. Selection of sampling points and conditions and sampling method

The sampling site is the Bagnon market during all campaigns.

We will carry out two random samplings on the market which has the most number of customers according to our observations.

At each sampling point, the same quantity of raw milk is taken (one and a half liter bottle). With the two samples taken, we will average the nutrients and microbiological analyses for each sample per campaign. Once the sample has been taken, it is imperative to put the samples in an isothermal enclosure (cooler) away from light.

In indeed, in the case of raw milk, light promotes the destruction of riboflavin (vitamin B2), the oxidation of vitamin C and carotenoid pigments, etc. **[34].**

In addition, the cooler to contain the samples must be thoroughly disinfected with bleach beforehand to avoid microbial contamination of raw milk.

The samples are then transported to the laboratory in the refrigerator at a temperature between 2 and 8°C. The ideal would be to perform the analyses the same day in the laboratory.

If this is not possible, the cold chain must not be interrupted during transport until the analyses which must be carried out as soon as possible (48 hours, exceptional circumstances). Refrigeration preserves the color, texture, and flavor (these organoleptic characteristics are related to nutrients) and slows the proliferation of bacteria and molds for a certain time **[1] [15].**

II.2.4. Campaign sheet

II.2.4.1. Viewing conditions

Viewing conditions	**Conditions before observation**
Wind : Bad ☐ Weak ☐ Medium ☐ Strong ☐ **Weather report :** Sunny dry weather ☐ Slightly cloudy ☐ Severely cloudy ☐ Humid weather ☐ Light rain ☐ Heavy rain ☐ **Notes:**	Light rain ☐……date …… Heavy rain☐ ……date …… Other conditions :

Table II.2. Observation conditions

II.2.4.2. Organoleptic characteristics of raw milk

Temperature (°C)	**Appearance and texture**	**pH**	**Dornic acdity**	**Color**	**Odor**	**Viscosity**	**Density**

Table II.3. Organoleptic characteristics of raw milk

II.2.4.3. Nutrients in raw milk

Water (g/L)	
Lactose (g/L)	
Lipids (g/L)	
Proteins (g/L)	
Minerals (g/L)	
Vitamins A and C characterization	
Notes	

Table II.4. Nutrients in raw milk

II.2.4.4. Microbiological analysis

Microscopic examinations :	
Brucella (Gram Stain)	
Mycoibacterium (Ziehl Neelsen Stain)	
Mesophilic aerobic germs (/L)	
Total Coliforms (/L)	
Indologenic and sulfhydrogenic bacteria (/L)	
Notes	

Table II.5. Microbiological analysis

II.3. Nutritional analyses

II.3.1. Physico-chemical and organoleptic analyses

II.3.1.1. Temperature

- Pour 20 ml of raw milk into a beaker, immerse the end of the thermometer and measure the temperature in °C.

II.3.1.2. Appearance and texture

- Pour the sample into a beaker and observe. It must be opaque to light.

II.3.1.3. Odor

- Take the beaker containing the milk for the temperature and smell. The smell must be related to the fat.

II.3.1.4. Color

- Take the beaker containing the milk for the temperature and observe the color. Normal color should be pearl white, note.

II.3.1.5. Viscosity

A falling-ball viscometer is used, which consists of a long vertical glass tube filled with milk, into which a spherical ball is dropped. The time required for the movement of the ball between fixed reference points A and B is measured. The viscosity of the milk is calculated according to the formula: $[2(ps-pl)ga^2]/9v$; Ps= solid density, pl: milk density, a=radius; g=9.8 $kg.m^{-2}$;v=speed ($m.s^{-1}$);$ps=2.8/Vs(4/3\pi a^2)$; a=0.035m; v=0.41/0.5. Milk viscosity=0.02N.s/m^2.

II.3.1.6. Density

- Take 10 ml or 1ml and weigh them on the electronic scale after taring it. Density = Weighed mass/10. The density must be between 1.032 and 1.035 at 25°C.

II.3.1.7. pH

- Pour 20 ml of the raw milk into a beaker, immerse the end of the pH meter and measure the pH. It should be 6.7 +/- 0.1.

II.3.1.8. Dornic acidity

II.3.1.8.1. Príniciple

The acidity of raw milk is measured by a strong base (sodium hydroxide in the presence of phenolphthalein). That acidity is practically only due to lactic acid according to the following reaction: CH_3-CHOH-COOH + NaOH $\longrightarrow$ CH_3-CHOH-COONa + H_2O.

II.3.1.8.2. Procedure

A test portion V_{milk} = 10 ml is neutralized with a sodium hydroxide solution of known concentration approximately equal to 100 mmol/L in the presence of phenolphthalein.

II.3.1.8.3. Expression of results

C_{H+} dosed = C_{NaOH}. V_{NaOH}/V_{milk}

The dosed H+ ions mainly come from lactic acid, hence: $C_{lactic\ acid} = C_{H+}$ dosed and $P_{lactic\ acid} = M_{lactic\ acid} . C_{lactic\ acid}$; M=90g/mol; 1° Dornic=1.10 mmol/L.

The Dornic acidity of raw cow's milk is between 16 and 18°.

II.3.1.9. Materials and reagents

- Beaker 250 ml
- Beaker 150 ml
- Thermometer
- pH meter and puffer solutions
- Viscosimeter
- Electronic scale : 0.00 – 500.00 g
- 10ml dropper
- 1ml dropper
- Cruet 25 ml
- Phenolphthalein
- Dornic NaOH solution 1/9 mol/L

II.3.2. Nutrients determination

II.3.2.1. Water (moisture)

II.3.2.1.1. Principle

Moisture content is an amount of water removed from raw milk during drying, denoted as a percentage of the weight. The major importance of moisture content lies in that this characteristic affects the dry matter content, and hence the yield of milk per mass unit. Typically, to determine the moisture content of milk an express drying method is used when weighed raw milk is dried in an electric oven at 130°C for 40 min. Repeatability of the results of moisture content determination by this method depends on the volume of raw milk and the height of its layer upon drying.

II.3.2.1.2. Procedure

- A sample of the row milk of 5 g weighted on the technical balances with an accuracy of 0.01 g should be placed into a weighting glass bottle with covers removed to be heated in an oven at 130°C.
- After 10 minutes, when the weighting bottles heat up to 130°C, the initial time is fixed. The duration of drying is 40 min.

Then weighing bottles are removed from a drying cabinet, covered with lids and cooled down in a desiccator for 15 ... 20 min, after which they are weighed on the technical balances with an accuracy of 0.01 g.

- The moisture content, *W* in %, is calculated by this formula below:

$$W = \frac{(a-b)}{(a-c)} 100,$$

Where: a is a mass of a weighing bottle with the sample before drying, g;
b is a mass of a weighing bottle with the sample after drying , g;
c is the mass of the empty weighing bottle, g.

The characteristic value of moisture content for row cow's milk is within the range from 82 and 89%.

II.3.2.2. Lactose

II.3.2.2.1. Principle

Reducing carbohydrates can be determined thanks to their reducing properties, in an alkaline medium and in hot conditions, with respect to 3-5 dinitrosalicylic acid (3,5-DNS) according to the following reaction: Reducing sugar + DNS (yellow) $\longrightarrow$ oxidized sugar + reduced DNS (orange-red). The reaction is non-stoichiometric, therefore dependent on the operating conditions. Consequently, we will operate by reference to a calibration range.

The yellow 3,5-DNS is reduced to orange-red 3-amino-5-nitrosalicylic acid, which can be measured colorimetrically at 530 - 540 nm.

II.3.2.2.2. Calibration range

Make a 1/50 dilution by pouring 2 ml of milk into a 100 ml flask and complete to the gauge mark. The spectrophotometric assay is carried out by carrying out a standard range of lactose at 2.00 g/L. The tubes of the range and the test tubes were prepared at the same time and as indicated in the table below:

Tubes	0	1	2	3	4	5	E1	E2
Lactose standard solution at 2.00g/L (mL)	0	0.4	0.6	0.8	1	1.2		
Distilled water (mL)	2	1.6	1.4	1.2	1	0.8		
Raw cow's milk (mL) (mL)							2	2
DNS reagent (mL) (mL)	1	1	1	1	1	1	1	1
	Homogenize and cap the tubes. Bring to a boiling water bath for exactly 5min Cool in a cold water bath then add:							
Distilled water (mL)	7	7	7	7	7	7	7	7
	Homogenize and leave to stand for 15min before reading the absorbance at 540nm against the reagent control							
Read absorbance								

Table II.6. Range of calibration and analysis

II.3.2.2.3. Spectrophotometer reading

Tubes	0	1	2	3	4	5	E1	E2
Masse of lactose in mg/tube	0	0.8	1.2	1.6	2	2.4	?	?
Absorbance at 530 - 540 nm								

Table II.7. Absorbance values

II.3.2.2.4. Curve plot: Abs = f (C)

The curve will give a line of type: y = ax + b

II.3.2.2.5. Calculation

- **Preparation of the calibration range (lactose solution at 2 g/L)**

Calculation of the amount of lactose in each tube = m (lac)/M (lac) = C (lac) x V(introduced)

We are : m(lac) = C(lac) x V(introduced) x M(lac) and rho(lac)=C(lac) x M(lac), so m(lac) = rho(lac) x V(lac) and n(lac) =m(lac)/M(lac)

n (lac)=[rho(lac) x V(introduced)]/M(lac)

Digital Application: tube n°1: n(lac)=[(2x0.4)/342]x1000 =2.339 µmol/tube

Or 2x0.4 = 0.8 mg/tube for the tube n° 1; 2x0.6 = 1.2 mg/tube for the tube 2, etc.

- **Dosage**

We are for an unknown concentration of lactose, we are a known absorbance. The line curve y= ax+b will give the concentration of lactose (mg/ml or g/L) in our milk according the formula: C (mg/tube) x50/2. Where 50 are the dilution factor and 2, the volume of milk in the tube.

The lactose content of raw cow's milk is 48g/L.

II.3.2.3. Lipids

II.3.2.3.1. Principle

Lipids react with sulfuric acid to form carbonium ions, which subsequently react with the vanillin phosphate ester to yield a purple complex that is measured photometrically.

II.3.2.3.2. Operating procedure

- **Treatment of raw milk and standard**

Products and reagents	Standard	Dosage
Total lipids standard at 8g/L	0.1	-
Raw milk	-	0.1
H_2SO_4	3	3

Table II.8. Treatment of milk and standard

- Shake well for 10 seconds and put in a boiling water bath for 10 minutes;
- Cool under tap water.

- **Color reaction**

Products and reagents (ml)	Blank	Standard	Dosage
Cold standard mixture	-	0.1	-
Cold milk mix	-	-	0.1
H_2SO_4	0.1	-	-
Sulpho-phospho-vanillic reagent	3	3	3

Table II.9. Color reaction

- Shake well for 10 seconds, avoiding air bubbles;
- Leave the color to develop for 30 minutes in the dark;
- Read the absorbance or OD at 530 nm.

II.3.2.3.3. Expression of results

Lipid concentration = (OD dosage/OD standard) x 8g/L

The lipid content of raw cow's milk is 37g/L.

II.3.2.4. Proteins

II.3.2.4.1. Principle

The “Lowry Assay: Protein by Folin Reaction” (Lowry et al., 1951) has been the most widely used method to estimate the amount of proteins (already in solution or easily-soluble in dilute alkali) in biological samples. First the proteins are pre-treated with copper ion in alkali solution, and then the aromatic amino acids in the treated sample reduce the phosphomolybdate phosphotungstic acid present in the Folin Reagent. The end product of this reaction has a blue color. The amount of proteins in the sample can be estimated via reading

the absorbance (at 750 nm) of the end product of the Folin reaction against a standard curve of a selected standard protein solution (in our case; Bovine serum Albumin-BSA).

II.3.2.4.2. Preparation of solutions

- **Solution A: 100 ml**

m Na_2CO_3 = 2g; m NaOH = 0.4g and water = sufficient quantity for 100ml

- **Solution B: 100 ml**

m$Na_2C_4H_4O_6$ =1g + 30ml H_2O + 0.5g $CuSO_4$, $5H_20$; water = sufficient quantity for 100ml

- **BSA solution**

m (BSA)=0.0025g + 10ml water (sufficient quantity for 10ml)=0.00025g/ml or 0.25mg/ml

- **Folin reagent : Folin and Ciocalteu's Phenol reagent**
- **Solution C :** 90ml Solution A + 1.8ml Solution B
- **Samples (**Two tubes/probe**)**

N probe	C, albumin (mg/ml)	V solution BSA (ml)	V H_2O (ml)	Samples (ml)	Total volume (ml)
1	0.063	0.1	0.3	-	0.4
2	0.125	0.2	0.2	-	0.4
3	0.188	0.3	0.1	-	0.4
4	0.250	0.4	0	-	0.4
5	0	0	0.4	-	0.4
Milk 1	0	0	0	0.4	0.4
Milk 2	0	0	0	0.4	0.4

Table II.10. Samples

- Add 2ml of Solution C in all steps, stir well and wait 10 minutes
- Add 2ml of Folin reagent in all steps, stir well and wait 30 minutes
- Milk 1 and milk 2 had been diluted at 1/100 (1ml in 100ml water)
- Measure the transmittance (T) or absorbance (A) against the probe 5 (tube 0) at 750nm through a spectrophotometer. A = 2 – log10 (%T)

Measure table

Probe	5	1	2	3	4	Milk 1	Milk 2
C	0	0.063	0.125	0.188	0.250	-	-
T%	-						
A	0						

Table II.11. Measures

- **Draw Curve A = f(C)**

By plotting the curve A = f(C) which gives a straight line of the type y = ax + b, from the measured values of A, we can plot the value of A of the milk on the curve and calculate the concentration of the proteins raw milk.

The proteins content of raw cow's milk is 34g/L.

II.3.2.5. Minerals: LAB23 I-MET006– Cendres brutes v11 2013-02-01-4/5

II.3.2.5.1. Principle

The sample is incinerated at 550°C. The residue is weighed.

II.3.2.5.2. Performance characteristics

The criteria for repeatability and reproducibility of application are the data included in the most recent version of the validation dossier.

II.3.2.5.3. Safety instructions and specific measures

Follow the safety instructions specified in the safety data sheets in the device logbook.

II.3.2.5.4. Method

- **Preparation of sample**

Sample is prepared according to procedure LAB23 P011 (Samples of Fertilizers and Feed)

- **Analysis: general case**

Weigh to the nearest 1 mg, about 5 g of the sample in a previously weighed incineration crucible. Place the crucible on the hot plate and gradually heat until the material is charred. Insert the crucible into the ventilated oven set at 550°C ± 5°C (see II.3.2.5.3). Maintain at this temperature at least 3 hours until white ash, light gray or reddish ash, apparently free of carbonaceous particles. Place the crucible in a desiccator, cool and weigh immediately.

- **Calculation and reporting**

Calculate the weight of the residue by deducting the tare. Express the result in% of the sample

$$\% = \frac{(w - t)}{p} x100$$

Where: w is the weight of the crucible and the ash after calcination (g),

t is the tare of the crucible (g),

p is the test portion (g).

The minerals content of raw cow's milk is 9g/L.

II.3.2.6. Vitamins

We are going to highlight a fat-soluble vitamin (Vitamin A) and a water-soluble vitamin (Vitamin C).

II.3.2.6.1. Vitamin A

II.3.2.6.1.1. Principle

Vitamin A is revealed by the Carr and Price reaction: a blue-violet color is obtained when a chloroform solution saturated with antimony trichloride is added to a chloroform solution of vitamin A. Unsaturated fatty acids can interfere with the reaction, it is necessary to work on the unsaponifiable. A discontinuous extraction is carried out.

II.3.2.6.1.2. Operating procedure

- **Saponifiable**

Introduce in a balloon:

- 10 ml of raw cow's milk;
- 20 ml of 50% (V/V) aqueous sodium hydroxide solution;
- 100 ml of ethanol;
- 2 ml of hydroquinone solution obtained by dissolving 20g in 100 ml of ethanol (reducing medium)

Bring to 90°C for 30 minutes.

- **Extraction of the unsaponifiable**

Pour the contents of the flask into a separating funnel and add:

- 100ml of water;
- 50 ml of diethyl oxide;
- Shake, and then add 50 ml of petroleum ether.
- Shake and let settle.
- Draw off and discard the lower aqueous phase.
- Extract again with 50 ml of petroleum ether.
- Wash the organic phase twice with 100 ml of water.
- Pour the organic phase into a beaker containing a little anhydrous sodium sulphate (desiccant). Leave in contact for 5 minutes.
- Filter. Evaporate the solvents in a water bath under a fume hood and concentrate until 1 ml is obtained.

- Dissolve the residue (unsaponifiable) in 3ml of chloroform.
- Transfer to a stoppered test tube.

- **Characterization**

- Pour a spatula tip of antimony trichloride into a test tube.
- Add 3 ml of chloroform (the solution must be saturated).
- Pour the chloroform solution of unsaponifiable into this tube drop by drop.
- Note the color obtained.

The presence of vitamin A is manifested by the appearance of a purple ring on contact with the chloroform solution saturated with antimony. If the medium has been completely dried out, the color changes to bright blue.

II.3.2.6.2. Vitamin C

II.3.2.6.2.1. Principle

To highlight vitamin C, the use of a solution of potassium permanganate (purple solution and powerful oxidant), may be required to react with vitamin C which is a reducing agent (antioxidant). When it reacts with vitamin C, it discolors according to the following reactions:

$2x \left[MnO_4^- + 8H^+ + 5e^- \longrightarrow Mn^{2+} + 4H_2O \right.$

$5x \left[\text{Vitamin C} \longrightarrow \text{DVit} + 2H^+ + 2e^- \right.$

$2MnO_4^-$ (purple) + $6H^+$ + 5Vitamin C $\longrightarrow$ $2Mn^{2+} + 8H_2O$ + DVit (colorless)

DVit : Dehydroascorbic acid

II.3.2.6.2.2. Procedure

- Prepare 100 ml of an acidified solution of potassium permanganate in water, at 1.2 g/L in a stoppered flask.
- Seek to detect vitamin C in our raw cow's milk using the potassium permanganate solution:

- Take 5 mL of potassium permanganate solution using a graduated cylinder; pour them into 3 test tubes, one of which will serve as a control.
- In the first test tube, pour orange juice using the dropper and count the drops until the potassium permanganate solution is discolored.
- In the second test tube, pour fresh cow's milk using the dropper and count the drops until the potassium permanganate solution is discolored.
- Compare the 3 three tubes: orange juice, raw cow's milk and control tube.
- Conclude on the vitamin C content of orange juice and raw cow's milk.

II.3.2.7. Equipment, materials, chemicals

II.3.2.7.1. Water

- Drying cabinet;
- Glass weighing bottles with lids;
- Crucible Tongs;
- Desiccator;
- A sample of milk;
- Technical balances.

II.3.2.7.2. Lactose

- Raw milk
- Clean Glassware
- Glass tubes: of any kind, approx. 10 ml
- Semi-micro cuvettes: Standard type plastic disposable cuvettes with 10 mm path length, sample capacity of 1.5-3 ml: different than the total carbohydrates measurements, we have to use these semi-micro cuvettes, since the volume of the end product we need to measure is only 1.3 ml.
- Water hot bath
- Water ice bath
- Parafilm
- Spectrophotometer: that goes up to 530 - 750 nm.
- Lactose solution.
- DNS Reagent
- Distilled water
- Other chemicals.

II.3.2.7.3. Lipids

- Spectrophotometer ;
- Water bath;
- Equipment for transferring volumes;
- Sulfuric acid ;
- Sulphophosphovanillic solution;
- Raw milk

II.3.2.7.4. Proteins

- Clean Glassware
- Glass tubes: of any kind, approx. 10 ml
- Semi-micro cuvettes: Standard type plastic disposable cuvettes with 10 mm path length, sample capacity of 1.5-3 ml: different than the total carbohydrates measurements, we have to use these semi-micro cuvettes, since the volume of the end product we need to measure is only 1.3 ml.
- Spectrophotometer: that goes up to 750 nm.
- Bovine Serum Albumine : Albumin bovine serum.
- Folin Reagent: Folin and Ciocalteu's Phenol Reagent, 2N.
- Sodium tartrat: Na2tartrat. 2(H_2O)
- Copper sulfate: $CuSO_4$, $5H_2O$.
- Other chemicals: NaOH, Na_2CO_3

II.3.2.7.5. Minerals

- Demineralized Water
- Analytical balance.
- Hotplate.
- Electric oven muffle (Heraeus), ventilated.
- Capsules in Opaline Quartz, diameter 75mm, height 28 mm.
- Filter paper (Schleicher & Schuell 5891 or equivalent) without ash.

II.3.2.7.6. Vitamins

II.3.2.7.6.1. Vitamin A

- Saponification balloon
- Water bath
- Test tubes of 25 ml, 50 ml, 100 ml.
- Equipment for volume transfer
- Separating funnel
- Beaker
- Pleated filter and funnel
- Test tubes
- Spatula

- Hood (windows covered with black paper)
- Raw cow's milk
- Aqueous solution of sodium hydroxide at 50% (V/V)
- Ethanol
- Hydroquinone solution (20 g of hydroquinone in 100 ml of ethanol)
- Diethyl oxide
- Petroleum ether
- Chloroform
- Antimony trichloride
- Anhydrous sodium sulfate.

II.3.2.7.6.2. Vitamin C

- 100 ml volumetric flask
- Balance
- Spatula
- Sulfuric acid
- Distilled water
- Potassium permanganate
- Raw cow's milk
- Test tubes.
- Tube rack.

II.4. Enumeration of microorganisms in foodstuffs

In addition to counting in solid medium and in liquid medium, we will also do research by microscopic examination (absence or presence).

II.4.1. Criteria

The criteria for microorganisms in raw cow's milk are as follows:

- **Mesophilic Aerobic Germs (MAGs): 10^6 germs/ml in solid medium;**
- **Coliforms: 10^2 germs/ml in liquid medium;**
- Faecal coliforms: 10 germs/ml in liquid medium;
- *E.coli*: 10 germs/ml in liquid medium;
- **Indologenic and putrid bacteria: 10^2 germs/ml in liquid medium;**
- Staphylococci: 10 germs/ml in solid medium;
- ***Brucella***, Salmonella: Absence/25g.

We will count and look for:

- Mesophilic aerobic germs (MAGs) as spoilage microorganisms;
- Total coliforms and indologenic and putrid bacteria as index or indicator microorganisms;
- *Brucella* and *Mycobacterium* as potentially pathogenic microorganisms.

II.4.2. Calculation of dilution intervals

We consider the satisfactory (**SAT**) and the corrupt (**COR**). We then refer to the official journal, we list the different microorganisms and their criteria, and then we look for the medium on which the germ must be cultivated.

This is actually the dilution interval we are looking for (**n**).

- For Mesophilic aerobic germs (MAGs), in solid medium, Petri dishes are considered to have between 30 and 300 colonies.

$30x10^{n}xS \leq 3N$: $\log 30 + \log 10^{n} + \log S \leq \log 3N$

$300x10nxS \geq 1000N$: $\log 300 + \log 10^{n} + \log S \geq \log 1000N$

S: Dilution factor exists for solid products or foods (10^1), but not for liquid products (it is equal to 10^0). Here, the milk will be diluted 1/10, as a precaution.

- For all other microorganisms in a solid medium, the number of colonies is considered to be between 15 and 150, 10 and 100, 5 and 50 or 3 and 30 and 1 in a liquid medium.

30: Minimum number of colonies; 10^{-n}: Dilution; 3N: SAT; 1000N: COR

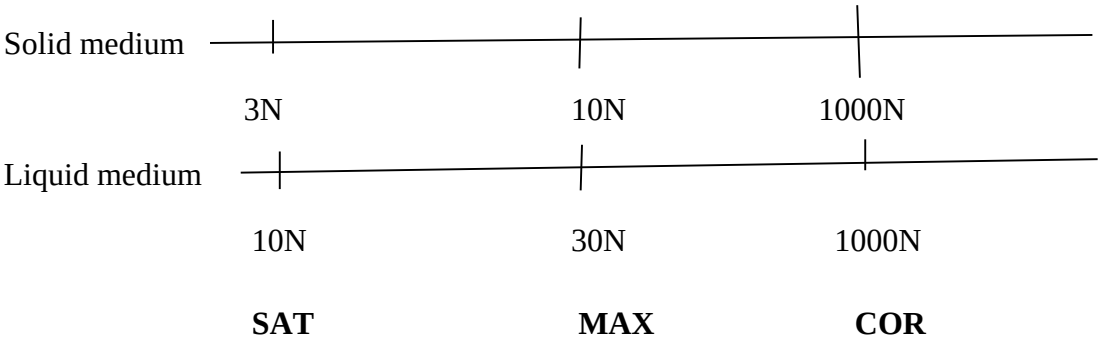

Figure II.2. Criteria scale for microorganisms

II.4.2.1. Mesophilic aerobic germs

N=10^6 germs/ml and SS =10^1 (milk diluted 1/10)

$30 .10^{n1}. S \leq 3N \Longrightarrow 30 .10^{n1}. 10^1 \leq 3.10^6$ **n1** $\leq$ **4**

$300.10n^2.S \geq 1000N \Longrightarrow 300.10^{n2}. 10^1 \geq 10^9$ **n2** $\geq$ **6** **Interval: [~~10^{-4}~~ ▶ 10^{-6}]**

II.4.2.2. Total coliforms and indologenic and putrid bacteria

N=10^2 germs/ml and S=10^1

$1 .10^{n1}. S \leq 10N \Longrightarrow 1 .10^{n1}. 10^1 \leq 10^3$ **n1** $\leq$ **2**

$1.10n^2.S \geq 1000N \Longrightarrow 1.10^{n2}. 10^1 \geq 10^95$ **n2** $\geq$ **4** **Interval: [$\underline{10^{-2}}$ ▶ 10^{-4}]**

For indologenic and putrid bacteria, we will take into account from raw milk (10^0) to 10^{-4}, to hope to have positive tubes.

II.4.2.3 .*Brucella* ; N=0 germ/25 g

Stamp stain typical of *Brucella* and similar to Ziehl Neelsen stain should be performed. But as the latter will be done for *Mycobacterium*, we are going to do a Gram stain for *Brucella*.

II.4.2.4. *Mycobacterium*; N=0 germ/25 g

A Ziehl Neelsen stain will be performed.

II.4.3. Analysis scheme

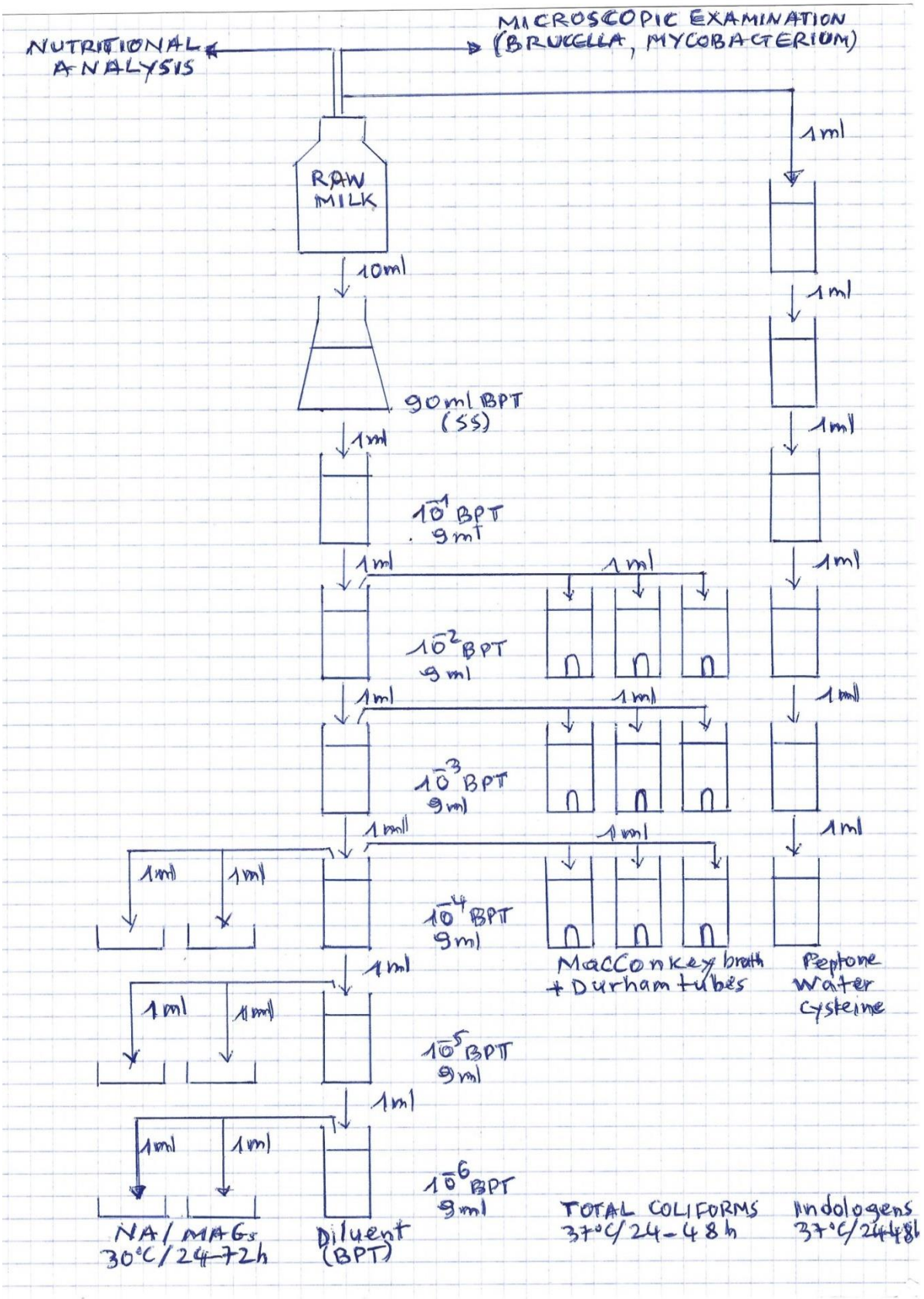

Figure II.3. Analysis Scheme

II.4.4. Equipment, materials, diluents, culture media, reagents

II.4.4.1. Equipment

- Memmert brand refrigerator for the conservation of the milk sample and the culture media;
- Memmert brand autoclave for the sterilization of culture media and diluents;
- Memmert Pasteur oven for sterilization of glassware (vial, test tubes, pipette, spreader);
- Latorius brand microscope for the observation of *Brucella* and *Mycobacterium*;
- Memmert oven, 30-37°C, for incubation of culture media.

II.4.4.2. Materials

- Milk sample (work material);
- 100 ml beaker for sampling;
- 02 Erlens of 100 ml including one for the stock solution (SS), one for the Nutrient Agar (NA) medium;
- 01 pipette of 10 ml to collect raw milk for SS;
- 12 graduated pipettes of 01 ml including 06 for the enumeration of MAGs, Coliforms and 06 for the search for indologenic and putrid bacteria;
- 06 cotton test tubes for successive dilutions;
- 15 screw cap tubes including 9 for MacConkey broth medium for total coliforms and 6 for peptone water medium with iron citrate for indologenic and putrid bacteria;
- 09 Durham tubes for MacConkey broth ;
- 06 Petri dishes for the MAGs.

II.4.4.3. Diluent

- 144 ml of Buffered peptone water (BPT) including 90 ml for SS and (09 ml X6) for the other successive dilutions.

II.4.4.4. Culture media

- 90 ml of NA medium for aerobic mesophilic germs;
- 90 ml of MacConkey broth for total coliforms;
- 60 ml peptone water with iron citrate for indologenic and putrid bacteria.

II.4.4.5. Reagents

II.4.4.5.1. For the count

- Kovac's reagent for the demonstration of indole production by indologenic bacteria.

II.4.4.5.2. For the detection of *Brucella* by Gram stain

- Crystal violet or gentian violet
- Lugol
- Ethanol
- Safranin
- Distilled water.

II.4.4.5.3. For the detection of Mycobacterium by Ziehl Neelsen staining

- Ziehl's carbol fuchsin
- Nitric acid or sulfuric acid
- Ethanol
- Distilled water
- Methylene blue.

II.4.5. Preparation of equipment, culture media, diluents, inoculation, reading

II.4.5.1. Preparation

- Sterilize the glass material (covered with Craft paper) in a Pasteur oven at 170°C for 1 hour;
- Prepare the diluents, the culture media (following the different preparation steps) as follows:
- Distribute 09 ml of diluent per cotton tube at the rate of 06 tubes and 90 ml of diluent in 01 Erlen of 100 ml, ie 144 ml of diluent;
- Distribute 10 ml of MacConkey broth/screw top tube, in the proportion of 09 tubes, ie 90 ml of medium;
- Distribute 10 ml of peptone water with iron citrate/screw top tube in the proportion of 06 tubes, i.e. 60 ml of medium;
- Pour 90 ml of prepared NA medium in a 100 ml Erlen flask stoppered with cotton;
- Group everything together in a metal basket, cover with craft paper;

- Sterilize diluents and culture media in an autoclave at 115-120°C for 15 to 20 minutes;
- Remove the equipment from the Pasteur oven and the diluents and culture media from the autoclave.
- Allow equipment, diluents and liquid culture media to cool;
- Keep the solid medium (NA) supercooled at 45-50°C in a water bath.

Note: Perform Gram stain for *Brucella* and Ziehl Neelsen stain for *Mycobacterium* while media and others are autoclaved or supercooled.

II.4.5.2. Inoculation

- Arrange the workstation around the Bunsen or Camping gas burner, in aseptic conditions throughout the handling;
- Take 1 ml of homogenized milk and quickly pour it into the first medium of peptone water with iron citrate which corresponds to the first dilution. For inoculate the successive dilutions, take a new pipette of 01 ml to inoculate the second homogenized tube from the first, and so on until the sixth tube of peptone water into iron citrate for indologenic and putrid bacteria;
- Take 10 ml of homogenized milk at laboratory temperature, and pour them in 90 ml of buffered peptone water diluent (stock solution: SS). The SS dilution is carried out, homogenize the solution;
- Take 01 ml of SS to pour it into 09 ml of BPT diluent to carry out the 10^{-1} dilution;
- Homogenize that 10^{-1} dilution, after throwing the previous pipette into the bleach tray;
- Take 01 ml of that dilution to pour it into 09 ml of BPT, to make the 10^{-2} dilution, homogenize it;
- Take with a 3rd pipette of 01 ml 3 times of the 10^{-2} dilution, to inoculate the 03 tubes of MacConkey broth corresponding to the 10^{-2} dilution, homogenize;
- Take 01 ml of that dilution to pour it into 09 ml of BPT, to make the 10^{-3} dilution, homogenize it;
- Take a 4th pipette of 01 ml 3 times of the 10^{-3} dilution, to inoculate the 03 tubes of MacConkey broth corresponding to the 10^{-3} dilution, homogenize;
- Take 01 ml of that dilution to pour it into 09 ml of BPT, to make the 10^{-4} dilution, homogenize it;

- Take with a 5th pipette of 01 ml 3 times of the 10^{-4} dilution, to inoculate the 03 tubes of MacConkey broth corresponding to the 10^{-4} dilution, homogenize, and 01 ml 2 times into the empty Petri dishes for NA , corresponding to the 10^{-4} dilution;
- Take 01 ml of that dilution to pour it into 09 ml of BPT, to make the 10^{-5} dilution, homogenize it;
- Take a 6th pipette of 01 ml 2 times of the 10^{-5} dilution, to inoculate the empty Petri dishes for NA, corresponding to the 10^{-5} dilution;
- Take 01 ml of that dilution to pour it into 09 ml of BPT, to make the 10^{-6} dilution, homogenize it;
- Take a seventh pipette of 01 ml 2 times of the 10^{-6} dilution, to inoculate the empty Petri dishes for NA, corresponding to the 10^{-6} dilution;
- Pour the NA into the 06 Petri dishes, at a rate of 15 ml per dish;
- Incubate the NA at 30°C for 24 to 72 hours and the other media (MacConkey broth, Pepton wter with iron citrate) at 37°C for 24 to 48 hours;
- Sterilize all the material used, wash it and put it away.

II.4.5.3. Screening for *Brucella* by Gram stain

II.4.5.3.1. Principle

The walls of Gram-negative bacteria have a high level of lipids (due to the outer membrane) and a thin layer of peptidoglycan. The alcohol in the bleach extracts the lipid, which makes the wall of Gram-negative bacteria more porous, and unable to retain the lugol violet complex, thereby bleaching the bacteria. The thicker peptidoglycan and higher degree of cross-linking traps the violet-lugol complex more efficiently, making the Gram-positive wall less susceptible to discoloration**.**

II.4.5.3.2. Procedure

- Take raw milk using a pipette and deposit it on a clean slide, under aseptic conditions;
- Spread the deposit on the slide to make a smear;
- Dry the slide over the Bunsen Burner or Camping flame without burning the preparation;
- Remove the blade and allow to cool;

- Flood air-dried, heat-fixed smear for 1 minute with crystal violet or gentian violet staining reagent. Please note that the quality of the smear (too heavy or too light cell concentration) will affect the staining results.
- Wash the blade in a gentle, indirect stream of tap water for 2 seconds
- Flooding with mordant: iodine or lugol. Wait 1 minute
- Wash the slide in a gentle, indirect stream of tap water for 2 seconds.
- Flood the blade with bleaching agent. Wait 15 seconds or add drop by drop to release bleaching agent
- Flood the slide with counterstain, 'safranin'. Wait 30 seconds to 1 minute.
- Wash the slide in a soft, indirect stream of tap water until no color appears in the effluent, then dry with paper towel.
- Observe the results of the oil immersion staining procedure. Examine under a microscope, objective x100

In the end, Gram negative bacteria will stain pink/red and Gram positive bacteria will stain blue/purple.

II.4.5.4. Detection of *Mycobacterium* by Ziehl Neelsen staining

II.4.5.4.1. Principle

The importance in pathology of the tubercle bacilli and the leprosy bacillus has led to the search for particular stains. They are based on the ability of these bacteria to retain, after hot staining with fuchsin, the pink color despite the action of a strong concentrated acid and 95% ethanol. This property is related to a particular constitution of the wall: it is very rich in lipids (mycolic acids, long-chain fatty acids and waxes) which are only found in these so-called "Alcohol-Acid-Resistant" or AAR bacteria (AARB).

II.4.5.4.2. Ziehl Neelsen staining procedure

- Make a smear and fix it. This smear should be neither too thin nor too thick;
- Heat carbol fuchsin for 10 minutes until vapors is released. The smear should not be dried out: add fuchsin during heating.
- Wash with distilled water then bleach with 1/3 nitric acid or 1/4 sulfuric acid for 5 minutes.
- Wash with distilled water then bleach with ethanol for 2 minutes.

- Wash with distilled water then stain with methylene blue for 5 minutes.
- Wash and let dry.
- Dry and observe by immersion under a microscope, x100 objective.

AARB appear pink while other microorganisms are blue.

II.4.5.5. Reading and expression of results

II.4.5.5.1. Mesophilic Aerobic Germs (MAGs)

Count between 30 and 300 colonies on the NA medium.

This involves counting the colonies that have appeared and expressing the result in CFU/g or CFU/ml of product analyzed on the basis of the formula:

$$N = \frac{\sum C}{(n1 + 0{,}1n2)V.d}$$

N = Number of germs in CFU/g or CFU/ml;

$\sum C$ = All the colonies counted for 2 successive dilutions giving colonies of 30 to 300 for the MAGs;

n1 = Number of dishes retained for the 1st dilution retained;

n2 = Number of dishes retained for the following dilution retained;

V = Volume of inoculum inoculated (ml);

d = 1st dilution retained.

Example:

Dilution	**Dishes (CFU)**		**Calculation**
10^{-1}	320	305	$\sum C = 42 + 38 = 80; n1 = 0 ; n2 = 2 ; V = 1ml;$ d= 10^{-2};
10^{-2}	38	42	N=80 / (0+0.1x2).1x10^{-2} = **4.10^{4} CFU/g**
10^{-3}	17	13	

Table II.12. Example of calculation

II.4.5.5.2. Total coliforms

Observe the turn of MacConkey Broth. All tubes that have turned from purple to yellow with gas production in Durham tubes are said to be positive, otherwise negative.

This is the 3-tubes system by dilution (most probable number method), NPP in French.

After incubation of the tubes, note for each test if the result is positive or negative. At each dilution, assign a number equal to the number of positive tubes.

- **Expression of results**

- Group to the number of 3 digits the sequence of digits obtained, starting with the digit obtained for the weakest dilution.
- Choose the largest number possible and if possible less than 330.
- Read the value of n from the table (see Annex 4 please).

n: most probable number of germs contained in the volume **V** in ml of the inoculum corresponding to the first digit of the combination.

The number of germs per ml or per g of food

$$= \frac{\frac{n}{V}:NPP}{valeur\ de\ la\ dilution\ correspondant\ au\ 1\ er\ chiffre}\ xSS$$

II.4.5.5.3. Indologenic and putrid bacteria

Indologenic bacteria are the bacteria that degrade protein tryptophan into indole. Sulfhydrogenic (or putrid) bacteria are bacteria that produce H_2S (hydrogen sulfide) from sulfur amino acids or mineral forms of sulfur (sulfate, sulfite, etc.).

On reading, we have:

- Red ring on the surface after addition of Kovac's reagent in the tube: presence in the inoculum of at least one indologenic bacterium.
- Black color in the tube: presence in the inoculum of at least one sulfhydrogenic bacterium.

After 48 hours of incubation at 37°C, the (I+S) index is determined by adding the number of indole+ tubes and the number of H_2S+ tubes.

- Index (I + S) < 3: good quality

- 3 < index (I + S) < 10: suspect foodstuff which must undergo a treatment equivalent to pasteurization.
- Index (I + S) > 10: withdrawal for legal reason "contamination by numerous microbial germs".

II.4.5.5.4. Screening for *Brucella* by Gram stain

Bacteria of the genus *Brucella* are very small Gram-negative Coccobacilli 0.5 to 0.7 µm in diameter and 0.5 to 1.5 µm in length (7.5 µm for a red blood cell). It is usually isolated rarely in pairs or in chains (in liquid medium) not encapsulated and not sporulated.

In the fresh state, they are animated by strong Brownian movements which can lead to the detection of a false mobility.

A dye characteristic linked to the acid resistance of the wall can be revealed by certain colorimetric techniques (Stamp, for example) allowing bacterioscopic diagnosis in veterinary medicine.

II.4.5.5.5. Detection of *Mycobacterium* by Ziehl Neelsen staining

AARB appear pink while other microorganisms are blue.

If examination of the smear reveals the presence of AARB, note a quantitative indication:

- More than one AARB per field, give the average number of bacilli per field by establishing the average over 10 to 20 microscopic fields;
- Less than one AARB per field, give the average number of bacilli per field by establishing the average out of 10.
- If the examination does not reveal any suspicious pattern recognition, “no AARB was observed” and specify the number of fields observed, which must be a maximum of 100.

Results of the nutritional analysis of raw cow's milk and the counting and research of the microorganisms contained in this milk are obtained. These will be discussed in the third part below.

III. THIRD PART: RESULTS AND DISCUSSION

III.1. RESULTS

III.1.1. Results of March 2022

III.1.1.1. Viewing conditions

Viewing conditions	**Conditions before observation**
Wind : Bad ☐ Weak ☐ X Medium☐ Strong☐ **Weather report :** Sunny dry weather ☐X Slightly cloudy X☐ Severely cloudy ☐ Humid weather ☐ Light rain ☐ X Heavy rain ☐ **Notes:**	Light rain ☐X……date 26 March 2022 Heavy rain☐ ……date …… Other conditions :

Table III.1. Observation conditions

- **Viewing conditions**

The temperature was 26°C at the date of 28 March 2022 at 6h51. It was a sunny dry weather although it rained on 26 March 2022. The weather was slightly cloudy. The humidity was 88%. The wind was weak and the speed was 8 km/h.

- **Conditions before observation**

It rained lightly on 26 March 2022.

III.1.1.2. Organoleptic characteristics of raw milk

Temperature (°C)	**Appearance and texture**	**pH**	**Dornic acidity (°D)**	**Color**	**Odor**	**Viscosity ($N.s/m^2$)**	**Density**
35	Opaque to light	6.4	27	Pearl white	Milk fats	-	1.02 at 25°C

Table III.2. Organoleptic characteristics of raw milk

- **Temperature**

The temperature of the raw milk was 35°C at the sampling.

- **Appearance and texture**

The milk was opaque to light. The criteria is the opacity to light. So, our raw milk met the appearance and texture criteria.

- **PH**

The pH of our raw milk was 6.4. The criteria should be 6.7 +/- 0.1. So, our milk didn't meet the criteria.

- **Dornic acidity**

C_{H+} dosed = C_{NaOH}. V_{NaOH}/V_{milk} CH^{+}= (0.1. 2.7)/10 = 0.027 mol/l or 27°D.

The Dornic acidity of raw cow's milk is between 16 and 18°. So, our milk didn't meet the criteria.

- **Color**

The milk was pearl white. The criteria is pearl white color. So, our raw milk met the color criteria.

- **Odor**

The milk had a typical odor of milk fat. The criteria is the odor of milk fats. So, our raw milk met the odor criteria.

- **Viscosity**

According to the formula, the Viscosity = $[2(12.7\text{-}1.02)9.8 \times (0/035)^2]/9x0.82$. We did not determined the viscosity due to a lack of material in the last minutes.

- **Density**

1 ml had been weighted and its mass was 1.02 g. So, the density of our raw milk was 1.02 at 25°C. The criteria is between 1.032 and 1.035 at 25°C. The density of our raw milk was lower than the criteria.

III.1.1.3. Nutrients in raw milk

Nutrients	Results	Criteria
Water (g/L)	**856.9 (85.69%)**	**820 – 890 (82 - 89%)**
Lactose (g/L)	**34**	**48**
Lipids (g/L)	**20**	**37**
Proteins (g/L)	**30**	**34**
Minerals (g/L)	**1.85**	**9**
Vitamins A and C characterization	**Presence**	**1**

Table III.3. Nutrients in raw milk

- **Water**

A glass bottle was weighed and the mass obtained was 46.35 g (c). A mass of raw milk, about 5 g was added to the watch glass and the mass obtained was 51.73 g (a). After 1 hour at 105°C in the oven, we obtained a mass of glass bottle and dehydrated milk of 47.12 g (b). After calculation according to the formula (a-b)/(a-c).100, the water content was 85.69% or 856.9 g/L. The criteria value of moisture content for raw cow's milk is within the range from 82 and 89%.

- **Lactose**

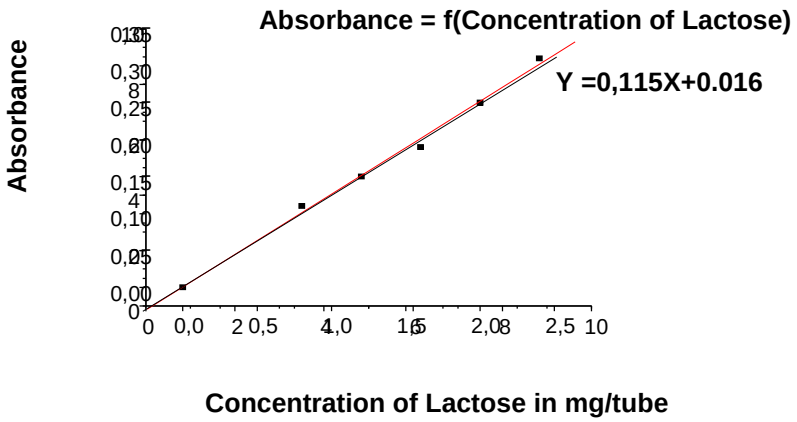

Figure III.1. Curve of lactose content determination

Calculation and reporting: $x = \frac{y-0.016}{0.115}$

On the curve, we had C=$\frac{0.108-0.016}{0.115}$= 0.80mg/tube. So, 0.80mg/tube x25=**2.0% or 20g/L.**
The lactose content of raw cow's milk was **48g/L**.

- **Lipids**

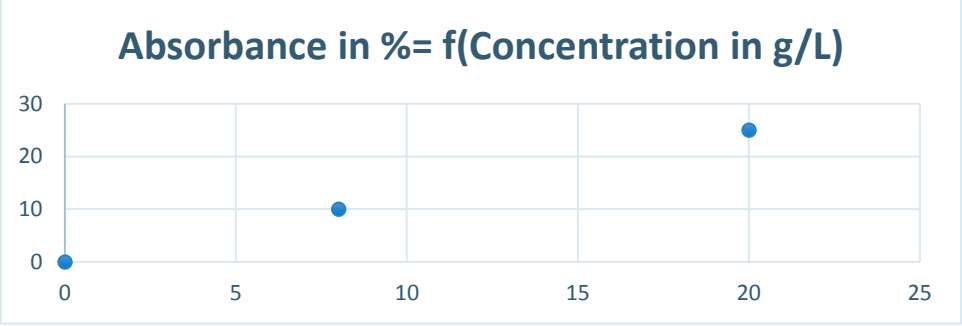

Figure III.2. Curve of lipids content determination

Lipid concentration = (OD dosage/OD standard) x 8g/L = (0.25/0.10)x8=**20g/L**.

The lipid content of raw cow's milk is **37g/L**.

- **Proteins**

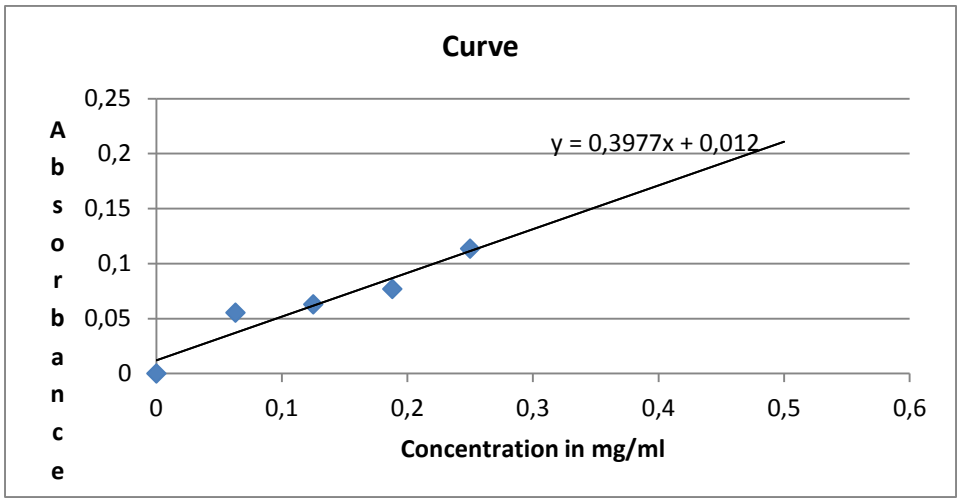

Figure III.3. Curve of proteins content determination

Calculation and reporting: $x = \frac{y-0.012}{0.397}$

On the curve, we had C=$\frac{0.1311-0.012}{0.397}$= 0,30mg/ml. So, 0.30mg/ml x100=**30g/L or 3.0%.**

- **Minerals**

A porcelain bucket was weighed and the mass obtained of the tare of the crucible was 16.29 g (t). A raw milk mass of 5.4 g (p) was added to the bucket mass and the mass obtained was 21.69 g (a). After 5 hours at 500°C in the oven, we obtained the weight of the crucible and the ash after calcination 16.30 g was (w). After calculation according to the formula (w-t)/(p).100, the ash content was 0.185% or 1.85g/L. The criteria of minerals content of raw cow's milk is 9g/L. The minerals content of our raw milk was lower than the criteria.

- **Vitamins A and C characterization**

- **Vitamin A**

The presence of vitamin A was manifested by the appearance of a purple ring on contact with the chloroform solution saturated with antimony. If the medium has been completely dried out, the color changed to bright blue.

- **Vitamin C**

- 5 mL of potassium permanganate solution were taken using a graduated cylinder; they were poured into 3 test tubes, one of which would serve as a control.
- In the first test tube, orange juice was poured using the dropper and count the drops until the potassium permanganate solution was discolored.
- In the second test tube, fresh cow's milk was poured using the dropper and count the drops until the potassium permanganate solution was discolored.
- The 3 three tubes were compared: orange juice, raw cow's milk and control tube.
- We concluded on the vitamin C content of orange juice and raw cow's milk.

III.1.1.4. Microbiological analysis

Microbiological analysis	**Results**	**Criteria**
Microscopic examinations :	**-**	**Absence/25g**
Brucella (Gram Stain)	**Probably presence**	**Absence/25g**
Mycoibacterium (Ziehl Neelsen Stain)	**Probably presence**	**Absence/25g**
Mesophilic aerobic germs (/ml)	**$4.6x10^7$**	**10^6**
Total Coliforms (/ml)	**$4.6x10^4$**	**10^2**
Indologenic and sulfhydrogenic bacteria (/ml)	**$\geq 10^6$**	**10^2**

Table III.4. Microbiological analysis

- **Microscopic examinations**

- ***Brucella* (Gram Stain)**

We observed the results of the oil immersion staining procedure. Examine under a microscope, objective x100.

In the end, Gram negative bacteria would stain pink/red and Gram positive bacteria would stain blue/purple. *Brucella* bacteria were Gram negative.

- ***Mycobacterium* (Ziehl Neelsen Stain)**

We washed and let dry.

We dried and observed by immersion under a microscope, x100 objective.

AARB appeared pink while other microorganisms were blue.

- **Mesophilic aerobic germs (/ml)**

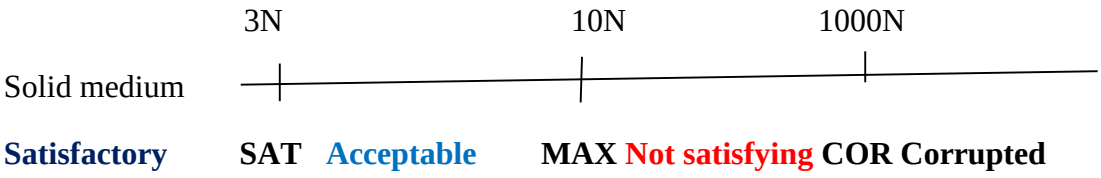

Figure III.4. Criteria scale for MAGs

Dilution	10^{-4}		10^{-5}		10^{-6}	
CFU/Petri dish	Unaccountable	Unaccountable	56	36	8	4

Table III.5. Results of MAGs counting

$$N = \frac{\sum C}{(n1 + 0{,}1n2)V.d}$$

$\sum C = 56 + 36 = 92; n1 = 2 ; n2 = 0 ; V = 1ml;$ d= 10^{-5}; N=92 / (2+0.1x0) x1x10^{-5} x 10= **$4.6x10^7$ CFU/ml.** The criteria is 10^6 CFU/ml. The criteria was 46 times ($4.6x10^7/10^6$) lower than the results of Mesophilic aerobic germs (MAGs) in our raw milk. So, our raw milk was not satisfying regarding MAGs.

- **Total Coliforms**

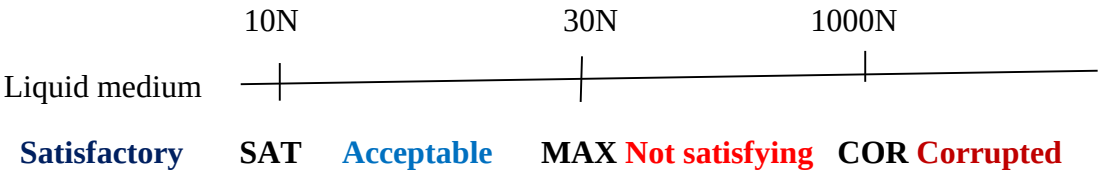

Figure III.5. Criteria scale for total coliforms counting

Dilution	10^{-2}			10^{-3}			10^{-4}		
Results in tubes	+	+	+	+	+	+	+	-	-
Total positive tubes	3			3			1		

Table III.6. Results of total coliforms counting

All the 6 tubes from dilution 10^{-2} to 10^{-3} were positive and only tube was positive at the dilution 10^{-4}. That corresponded to 331, ie 46 on the MacGrady's table. Therefore, we would have $46.10^2.10$ = **$4.6x10^4$ Total Coliforms/ml**. The criteria is 10^2 germs /ml. That meaned, we had 460 times higher than the criterion. So, our raw milk was not satisfying regarding « total coliforms ».

- **Indologenic and sulfhydrogenic bacteria**

Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
H_2S (S)	+	+	+	+	+	+
Indole (I)	+	+	+	+	+	+

Table III.7. Results of indologenic and sulfhydrogenic bacteria counting

I = 6 positive tubes; S = 6 positive tubes; I + S = 12. So, Index (I + S) > 10: our raw milk was withdrawal for legal reason "contamination by numerous microbial germs". **N ≥ 10^6 germs/ml.** It was 10,000 times ($10^6/10^2$) higher than the criterion.

III.1.2. Results of June 2022

III.1.2.1. Viewing conditions

Viewing conditions	**Conditions before observation**
Wind : Bad ☐ Weak ☐ Medium ☐X Strong☐ **Weather report :** Sunny dry weather ☐ Slightly cloudy X ☐ Severely cloudy ☐ Humid weather ☐ Light rain ☐X Heavy rain ☐ **Notes:**	Light rain ☐X date 28 may 2022…… Heavy rain☐ ……date …… Other conditions :

Table III.8. Observation conditions

- **Viewing conditions**

The temperature was 24°C at the date of 30 May 2022 at 6h46. It was a sunny dry weather although it rained on 28 May 2022. The weather was slightly cloudy. The humidity was 79%. The wind was weak and the speed was 16 km/h.

- **Conditions before observation**

It rained lightly on 28 May 2022.

III.1.2.2. Organoleptic characteristics of raw milk

Tempe-rature (°C)	**Appearance and texture**	**pH**	**Dornic acidity (°D)**	**Color**	**Odor**	**Density**
34.5	Opaque to light	6.31	28	Pearl white	Milk fats	1.13 at 25°C

Table III.9. Organoleptic characteristics of raw milk

- **Temperature**

The temperature was 34.5°C at the sampling.

- **Appearance and texture**

The milk was opaque to light. The criteria is the opacity to light. So, our raw milk met the appearance and texture criteria.

- **PH**

The pH of our raw milk was 6.31. The criteria should be 6.7 +/- 0.1. So, our milk didn't meet the criteria.

- **Dornic acidity**

C_{H+} dosed = C_{NaOH}. V_{NaOH}/V_{milk} CH^{+}= (0.1 2.8)/10 = 0.031 mol/l or 28°D

The dosed H+ ions mainly come from lactic acid, hence: $C_{lactic\ acid} = C_{H+}$ dosed and $P_{lactic\ acid} = M_{lactic\ acid} . C_{lactic\ acid}$; M=90g/mol; 1° Dornic=1.10 mmol/L.

The criteria of Dornic acidity of raw cow's milk is between 16 and 18°. So, our milk didn't meet the criteria.

- **Color**

The milk was white. The criteria is the white color. So, our raw milk met the color criteria.

- **Odor**

The milk had a typical odor of milk fat. The criteria is the odor of milk fats. So, our raw milk met the odor criteria.

- **Density**

1 ml had been weighted and its mass was 1.13 g. So, the density of our raw milk was 1.13 at 25°C. The criteria is ≥ 1.032 at 25°C. The density of our raw milk was higher than the criteria.

III.1.2.3. Nutrients in raw milk

Nutrients	Results	Criteria
Water (g/L)	**836.9 (83.69%)**	**820 – 890 (82 - 89%)**
Lactose (g/L)	**32**	**48**
Lipids (g/L)	**21**	**37**
Proteins (g/L)	**28**	**34**
Minerals (g/L)	**2.03**	**9**
Vitamins A and C characterization	**Presence**	**1**

Table III.10. Nutrients in raw milk

- **Water**

A glass bottle was weighed and the mass obtained was 46.19 g (c). A mass of raw milk, about 5 g was added to the watch glass and the mass obtained was 51.40 g (a). After 1 hour at 105°C in the oven, we obtained a mass of glass bottle and dehydrated milk of 47.04 g (b). After calculation according to the formula (a-b)/(a-c).100, the water content was 83.69% or 836.9 g/L.

The criteria value of moisture content for raw cow's milk is within the range from 82 and 89%.

- **Lactose**

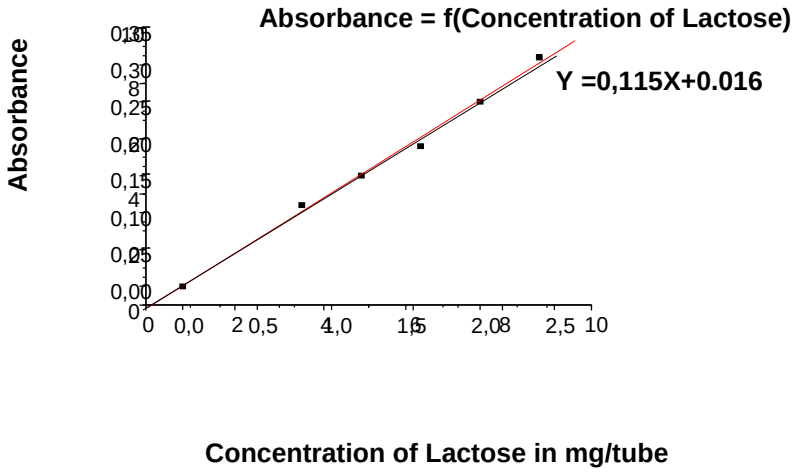

Figure III.6. Curve of lactose content determination

Calculation and reporting: $x = \frac{y-0.016}{0.115}$

On the curve, we had C= $\frac{0.1448-0.016}{0.115}$= 1.12mg/tube. So, 1.12mg/tube x25=**2.8% or 28g/L.**
The lactose content of raw cow's milk was **48g/L**.

- **Lipids**

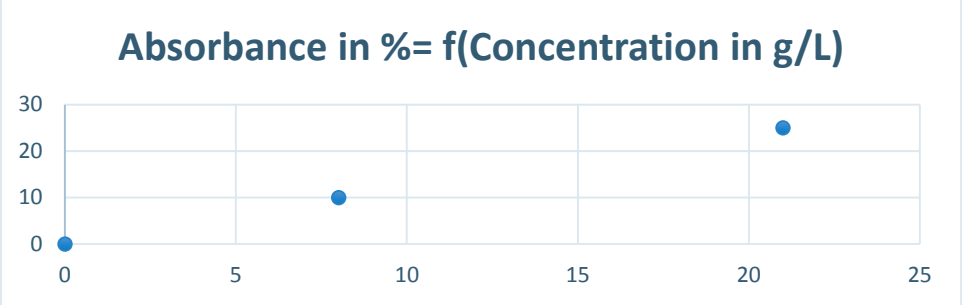

Figure III.7. Curve of lipids content determination

Lipid concentration = (OD dosage/OD standard) x 8g/L = (0.2625/0.10)x8=**21g/L**.

The lipid content of raw cow's milk was **37g/L**.

- **Proteins**

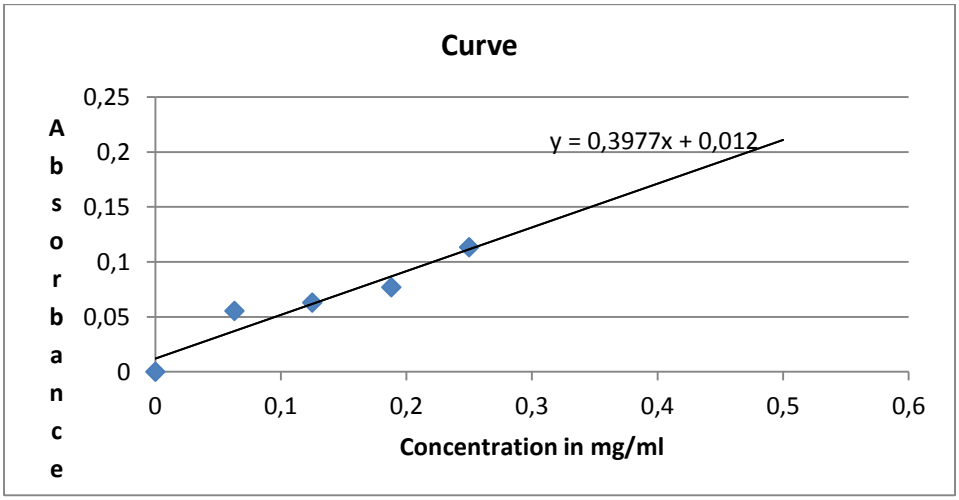

Figure III.8. Curve of proteins content determination

Calculation and reporting: $x = \frac{y-0.012}{0.397}$

On the curve, we had C=$\frac{0.1232-0.012}{0.397}$= 0,28mg/ml. So, 0.28mg/ml x100=**28 g/L** or **2.8%.**

- **Minerals**

A porcelain bucket was weighed and the mass obtained of the tare of the crucible was 17.35 g (t). A raw milk mass of 5.90 g (p) was added to the bucket mass and the mass obtained was 23.25 g (a). After 5 hours at 500°C in the oven, we obtained the weight of the crucible and the ash after calcination 17.37 g was (w). After calculation according to the formula (w-t)/(p).100,

the ash content was **0.338%** or **3.38g/L**. The minerals content of raw cow's milk is 9g/L. The minerals content of our raw milk was higher than the criteria.

- **Vitamins A and C characterization**

- **Vitamin A**

The presence of vitamin A was manifested by the appearance of a purple ring on contact with the chloroform solution saturated with antimony. If the medium has been completely dried out, the color changes to bright blue.

- **Vitamin C**

- 5 mL of potassium permanganate solution were taken using a graduated cylinder; they were poured into 3 test tubes, one of which would serve as a control.
- In the first test tube, orange juice was poured using the dropper and count the drops until the potassium permanganate solution was discolored.
- In the second test tube, fresh cow's milk was poured using the dropper and count the drops until the potassium permanganate solution was discolored.
- We compared the 3 three tubes: orange juice, raw cow's milk and control tube.
- We concluded on the vitamin C content of orange juice and raw cow's milk.

III.1.2.4. Microbiological analysis

Microbiological analysis	**Results**	**Criteria**
Microscopic examinations :	**-**	**Absence/25g**
***Brucella* (Gram Stain)**	**Probably presence**	**Absence/25g**
***Mycoibacterium* (Ziehl Neelsen Stain)**	**Probably presence**	**Absence/25g**
Mesophilic aerobic germs (/ml)	**$5.2x10^7$**	**10^6**
Total Coliforms (/ml)	**$1.1x10^5$**	**10^2**
Indologenic and sulfhydrogenic bacteria (/ml)	**$\geq 10^6$**	**10^2**

Table III.11. Microbiological analysis

- **Microscopic examinations**

- ***Brucella* (Gram Stain)**

We observed the results of the oil immersion staining procedure. Examined under a microscope, objective x100.

In the end, Gram negative bacteria would stain pink/red and Gram positive bacteria would stain blue/purple. *Brucella* bacteria were Gram negative.

- ***Mycobacterium* (Ziehl Neelsen Stain)**

We washed and let dry.

We dried and observed by immersion under a microscope, x100 objective.

AARB appeared pink while other microorganisms were blue.

- **Mesophilic aerobic germs (/ml)**

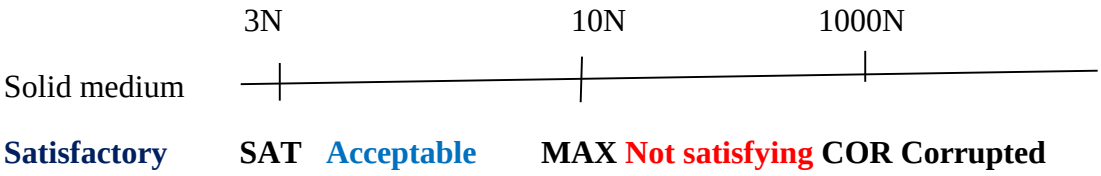

Figure III.9. Criteria scale for MAGs

Dilution	10^{-4}		10^{-5}		10^{-6}	
CFU/Petri dish	Unaccountable	Unaccountable	58	46	7	5

Table III.12. Results of MAGs counting

$$N = \frac{\sum C}{(n1 + 0{,}1n2)V.d}$$

$\sum C = 58 + 46 = 104; n1 = 2 ; n2 = 0 ; V = 1ml$; d= 10^{-5}; N=104 / (2+0.1x0) x1x10^{-5} x 10= **5.2x10^{7} CFU/ml.** The criteria is 10^{6} CFU/ml. The criteria was 52 times (5.2x10^{7}/10^{6}) lower than the results of Mesophilic aerobic germs (MAGs) in our raw milk. So, our raw milk was not satisfying regarding MAGs.

- **Total Coliforms**

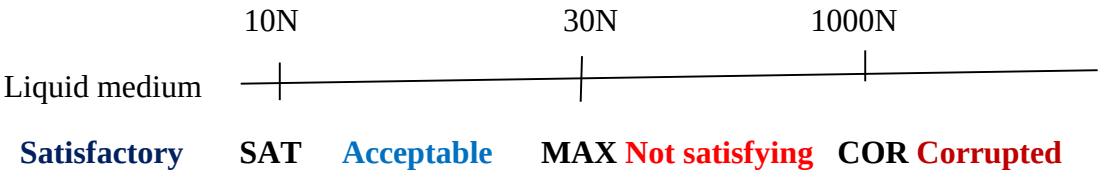

Figure III.10. Criteria scale for total coliforms

Dilution	10^{-2}			10^{-3}			10^{-4}		
Results in tubes	+	+	+	+	+	+	+	+	-
Total positive tubes	3			3			2		

Table III.13. Results of total coliforms counting

All the 6 tubes from dilution 10^{-2} to 10^{-3} were positive and only a tube was positive at the dilution 10^{-4}. That corresponded to 332, ie 110 on the MacGrady's table. Therefore, we had $110.10^2.10$ = **$1.1x10^5$ Total Coliforms/ml**. The criteria is 10^2 germs /ml. That meaned, we had 1,100 times higher than the criterion. So, our raw milk was corrupted regarding « total coliforms ».

- **Indologenic and sulfhydrogenic bacteria**

Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
H_2S (S)	+	+	+	+	+	+
Indole (I)	+	+	+	+	+	+

Table III.14. Results of indologenic and sulfhydrogenic bacteria counting

I = 6 positive tubes; S = 6 positive tubes; I + S = 12.

So, Index (I + S) > 10: our raw milk was withdrawal for legal reason "contamination by numerous microbial germs". **N ≥ 10^6 germs/ml.** It was 10,000 times ($10^6/10^2$) higher than the criterion.

III.1.3. Results of September 2022

III.1.3.1. Viewing conditions

Viewing conditions	**Conditions before observation**
Wind : Bad ☐ Weak ☐X Medium☐ Strong☐ **Weather report :** Sunny dry weather ☐ X Slightly cloudy ☐ Severely cloudy ☐ Humid weather ☐ Light rain ☐ X Heavy rain ☐ **Notes:**	Light rain ☐X……date 27 September 2022 Heavy rain☐ ……date …… Other conditions :

Table III.15. Observation conditions

- **Viewing conditions**

The temperature was 28°C at the date of 27 September 2022 at 7h01. It was a sunny dry weather although it rained on 27 September 2022. The weather was slightly cloudy. The humidity was 88%. The wind was weak and the speed was 8 km/h.

- **Conditions before observation**

It rained lightly on 27 September 2022.

III.1.3.2. Organoleptic characteristics of raw milk

Temperature (°C)	Appearance and texture	pH	Dornic acidity (°D)	Color	Odor	Density
35.5	Opaque to light	6.33	30	Pearl white	Milk fats	1.02 at 25°C

Table III.16. Organoleptic characteristics of raw milk

- **Temperature**

The temperature was 28°C at the sampling.

- **Appearance and texture**

The milk is opaque to light. The criteria is the opacity to light. So, our raw milk meets the appearance and texture criteria.

- **PH**

The pH of our raw milk was 6.33. The criteria should be 6.7 +/- 0.1. So, our milk doesn't meet the criteria.

- **Dornic acidity**

C_{H+} dosed = C_{NaOH}. V_{NaOH}/V_{milk} CH^{+}= (0.1 x3)/10 = 0.030 mol/l or 30°D

The dosed H+ ions mainly come from lactic acid, hence: $C_{lactic\ acid} = C_{H+}$ dosed and $P_{lactic\ acid} = M_{lactic\ acid} . C_{lactic\ acid}$; M=90g/mol; 1° Dornic=1.11 mmol/L.

The criteria of Dornic acidity of raw cow's milk is between 16 and 18°. So, our milk didn't meet the criteria.

- **Color**

The milk was white. The criteria is the white color. So, our raw milk met the color criteria.

- **Odor**

The milk had a typical odor of milk fat. The criteria is the odor of milk fats. So, our raw milk met the odor criteria.

- **Density**

1 ml had been weighted and its mass was 1.02 g. So, the density of our raw milk was 1.02 at 25°C. The criteria is ≤ 1.032 at 25°C. The density of our raw milk was lower than the criteria.

III.1.3.3. Nutrients in raw milk

Nutrients	Results	Criteria
Water (g/L)	**829.1 (82.91%)**	**820 – 890 (82 - 89%)**
Lactose (g/L)	**32**	**48**
Lipids (g/L)	**23**	**37**
Proteins (g/L)	**30**	**34**
Minerals (g/L)	**3.82**	**9**
Vitamins A and C characterization	**Presence**	**1**

Table III.17. Nutrients in raw milk

- **Water**

A glass bottle was weighed and the mass obtained was 42.24 g (c). A mass of raw milk, about 5 g was added to the watch glass and the mass obtained was 47.33 g (a). After 1 hour at 105°C in the oven, we obtained a mass of glass bottle and dehydrated milk of 43.11 g (b). After calculation according to the formula (a-b)/(a-c).100, the water content was 82.91% or 829.1 g/L. The criteria value of moisture content for raw cow's milk is within the range from 82 and 89%.

- **Lactose**

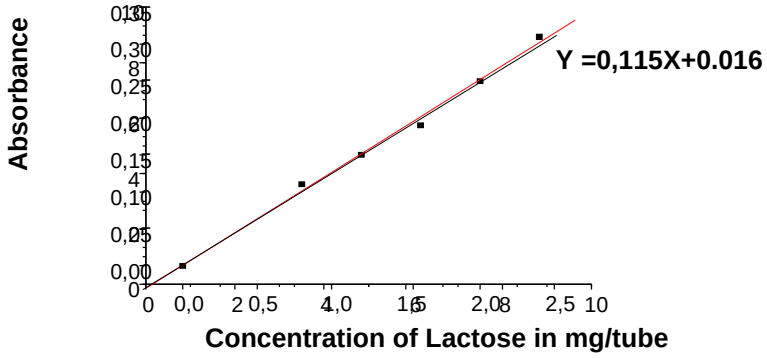

Figure III.11. Curve of lactose content determination

Calculation and reporting: $= \frac{y-0.016}{0.115}$ **;** On the curve, we had C= $\frac{0.1632-0.016}{0.115}$ = 1.28mg/tube. So, 1.28mg/tube x25=**3.2% or 32g/L.**

The lactose content of raw cow's milk was **48g/L.**

- **Lipids**

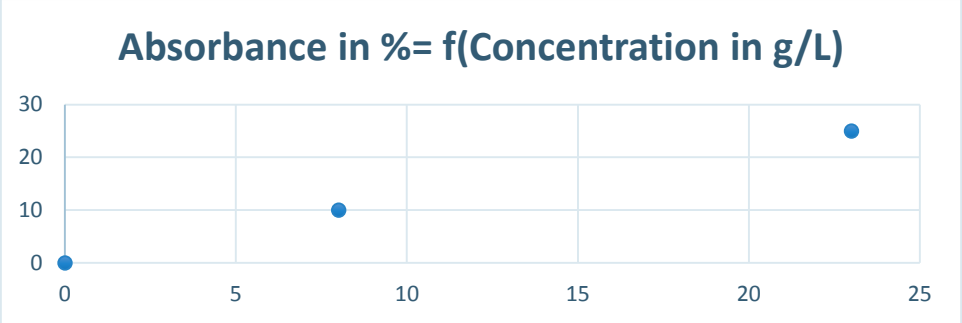

Figure III.12. Curve of lipids content determination

Lipid concentration = (OD dosage/OD standard) x 8g/L= (0.2875/0.10)x8=**23g/L**.

The lipid content of raw cow's milk was 37g/L.

- **Proteins**

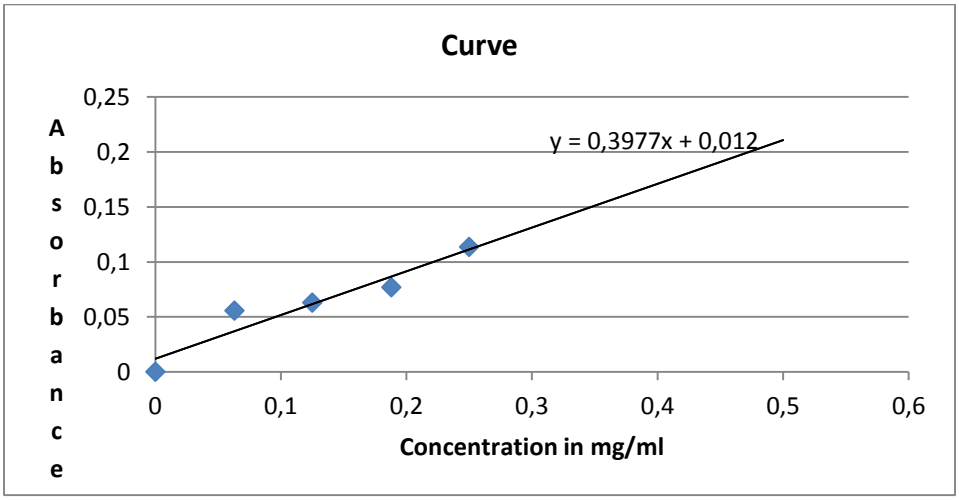

Figure III.13. Curve of proteins content determination

Calculation and reporting: $x = \frac{y-0.012}{0.397}$

On the curve, we had C=$\frac{0.1311-0.012}{0.397}$= 0,30mg/ml. So, 0.30mg/ml x100=**30g/L or 3.0%.**

- **Minerals**

A porcelain bucket was weighed and the mass obtained of the tare of the crucible was 15.84 g (t). A raw milk mass of 5.96 g (p) was added to the bucket mass and the mass obtained was 21.08 g (a). After 5 hours at 500°C in the oven, we obtained the weight of the crucible and the ash after calcination 15.86 g was (w). After calculation according to the formula (w-t)/(p).100,

the ash content was 0.382% or **3.82g/L**. The criteria of minerals content of raw cow's milk is 9g/L. The minerals content of our raw milk was higher than the criteria.

- **Vitamins A and C characterization**

- **Vitamin A**

The presence of vitamin A was manifested by the appearance of a purple ring on contact with the chloroform solution saturated with antimony. If the medium has been completely dried out, the color changed to bright blue.

- **Vitamin C**

- 5 mL of potassium permanganate solution were taken using a graduated cylinder; they were poured into 3 test tubes, one of which served as a control.
- In the first test tube, orange juice was poured using the dropper and count the drops until the potassium permanganate solution was discolored.
- In the second test tube, fresh cow's milk was poured using the dropper and count the drops until the potassium permanganate solution was discolored.
- The 3 three tubes were compared: orange juice, raw cow's milk and control tube.
- We concluded on the vitamin C content of orange juice and raw cow's milk.

III.1.3.4. Microbiological analysis

Microbiological analysis	**Results**	**Criteria**
Microscopic examinations :	**-**	**Absence/25g**
***Brucella* (Gram Stain)**	**Probably presence**	**Absence/25g**
***Mycoibacterium* (Ziehl Neelsen Stain)**	**Probably presence**	**Absence/25g**
Mesophilic aerobic germs (/ml)	**$7.0x10^7$**	**10^6**
Total Coliforms (/ml)	**$1.1x10^5$**	**10^2**
Indologenic and sulfhydrogenic bacteria (/ml)	**$\geq 10^6$**	**10^2**

Table III.18. Microbiological analysis

- **Microscopic examinations**

- ***Brucella* (Gram Stain)**

The results of the oil immersion staining procedure were observed, examined under a microscope, objective x100.

In the end, Gram negative bacteria would stain pink/red and Gram positive bacteria would stain blue/purple. *Brucella* bacteria were Gram negative.

- ***Mycobacterium* (Ziehl Neelsen Stain)**

We washed and let dry.

We dried and observed by immersion under a microscope, x100 objective.

AARB appeared pink while other microorganisms were blue.

- **Mesophilic aerobic germs (/ml)**

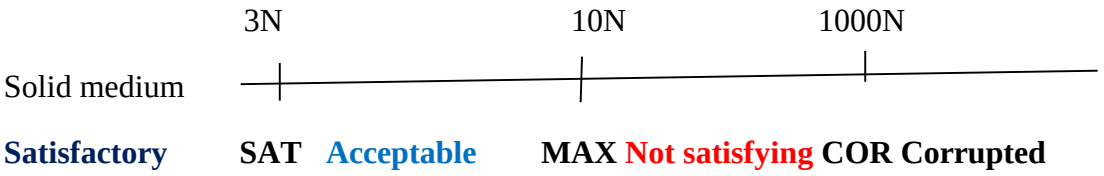

Figure III.14. Criteria scale for MAGs

Dilution	10^{-4}		10^{-5}		10^{-6}	
CFU/Petri dish	Unaccountable	Unaccountable	74	65	10	7

Table III.19. Results of MAGs counting

$$N = \frac{\sum C}{(n1 + 0{,}1n2)V.d}$$

$\sum C = 74 + 65 = 139; n1 = 2 ; n2 = 0 ; V = 1ml$; d= 10^{-5}; N=139 / (2+0.1x0) x1x10^{-5} x 10= **$7.0x10^7$ CFU/ml.** The criteria is 10^6 CFU/ml. The criteria was 70 times ($7.0x10^7/10^6$) lower than the results of Mesophilic aerobic germs (MAGs) in our raw milk. So, our raw milk was not satisfying regarding MAGs.

- **Total Coliforms**

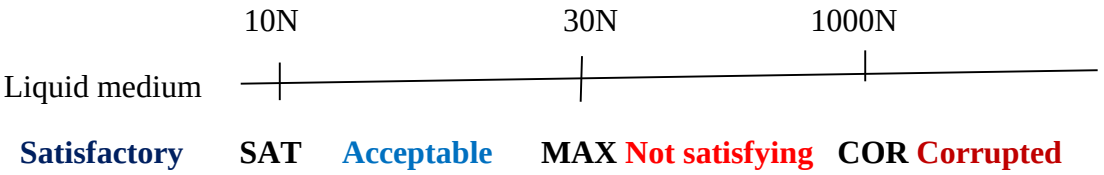

Figure III.15. Criteria scale for total coliforms

Dilution	10^{-2}			10^{-3}			10^{-4}		
Results in tubes	+	+	+	+	+	+	+	+	-
Total positive tubes	3			3			2		

Table III.20. Results of total coliforms counting

All the 6 tubes from dilution 10^{-2} to 10^{-3} were positive and only two tubes were positive at the dilution 10^{-4}. That corresponded to 332, ie 110 on the MacGrady's table. Therefore, we would have $110.10^2.10$ = **$1.1x10^5$ Total Coliforms/ml**. The criteria is 10^2 germs /ml. That meaned, we had 1,100 times higher than the criterion. So, our raw milk was corrupted regarding « total coliforms ».

- **Indologenic and sulfhydrogenic bacteria**

Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
H_2S (S)	+	+	+	+	+	+
Indole (I)	+	+	+	+	+	+

Table III.21. Results of indologenic and sulfhydrogenic bacteria counting

I = 6 positive tubes; S = 6 positive tubes; I + S = 12.

So, Index (I + S) > 10: our raw milk was withdrawal for legal reason "contamination by numerous microbial germs". **N ≥ 10^6 germs/ml.** It was 10,000 times ($10^6/10^2$) higher than the criterion.

III.1.4. Results of December 2022

III.1.4.1. Viewing conditions

Viewing conditions	**Conditions before observation**
Wind : Bad ☐ Weak ☐ X Medium☐ Strong☐ **Weather report :** Sunny dry weather ☐X Slightly cloudy ☐ Severely cloudy ☐ Humid weather ☐ Light rain ☐X Heavy rain ☐ **Notes:**	Light rain ..☐X…date 1st December 2022…. Heavy rain☐ ……date ……. Other conditions :

Table III.22. Observation conditions

- **Viewing conditions**

The temperature was 29°C at the date of 1st December 2022 at 7h11. It was a sunny dry weather although it rained lightly on 30 November 2022. The weather was slightly cloudy. The humidity was 80%. The wind was weak and the speed was 14 km/h.

- **Conditions before observation**

It rained lightly on 30 November 2022.

III.1.4.2. Organoleptic characteristics of raw milk

Temperature (°C)	Appearance and texture	pH	Dornic acidity (°D)	Color	Odor	Density
33	Opaque to light	6.4	25	Pearl white	Milk fat	1.03 at 25°C

Table III.23. Organoleptic characteristics of raw milk

- **Temperature**

The temperature was 33°C at the sampling.

- **Appearance and texture**

The milk was opaque to light. The criteria is the opacity to light. So, our raw milk met the appearance and texture criteria.

- **PH**

The pH of our raw milk was 6.4. The criteria should be 6.7 +/- 0.1. So, our milk didn't meet the criteria.

- **Dornic acidity**

C_{H+} dosed = C_{NaOH}. V_{NaOH}/V_{milk} CH^{+}= (0.1x 2.5)/10 = 0.25 mol/l or 25°D

The dosed H+ ions mainly come from lactic acid, hence: $C_{lactic\ acid} = C_{H+}$ dosed and $P_{lactic\ acid} = M_{lactic\ acid} . C_{lactic\ acid}$; M=90g/mol; 1° Dornic=1.11 mmol/L.

The criteria of Dornic acidity of raw cow's milk is between 16 and 18°. So, our milk didn't meet the criteria.

- **Color**

The milk was white. The criteria is the white color. So, our raw milk met the color criteria.

- **Odor**

The milk had a typical odor of milk fat. The criteria is the odor of milk fats. So, our raw milk met the odor criteria.

- **Density**

1 ml had been weighted and its mass was 1.03 g. So, the density of our raw milk was 1.03 at 25°C. The criteria is ≤ 1.032 at 25°C. The density of our raw milk was lower than the criteria.

III.1.4.3. Nutrients in raw milk

Nutrients	Results	Criteria
Water (g/L)	**843.2 (84.32%)**	**820 – 890 (82 - 89%)**
Lactose (g/L)	**30**	**48**
Lipids (g/L)	**20**	**37**
Proteins (g/L)	**30**	**34**
Minerals (g/L)	**2.30**	**9**
Vitamins A and C characterization	**Presence**	**1**

Table III.24. Nutrients in raw milk

- **Water**

A glass bottle was weighed and the mass obtained was 44.20 g (c). A mass of raw milk, about 5 g was added to the watch glass and the mass obtained was 49.30 g (a). After 1 hour at 105°C in the oven, we obtained a mass of glass bottle and dehydrated milk of 44.99 g (b). After calculation according to the formula (a-b)/(a-c).100, the water content was 84.32% or 843.2 g/L. The criteria value of moisture content for raw cow's milk is within the range from 82 and 89%.

- **Lactose**

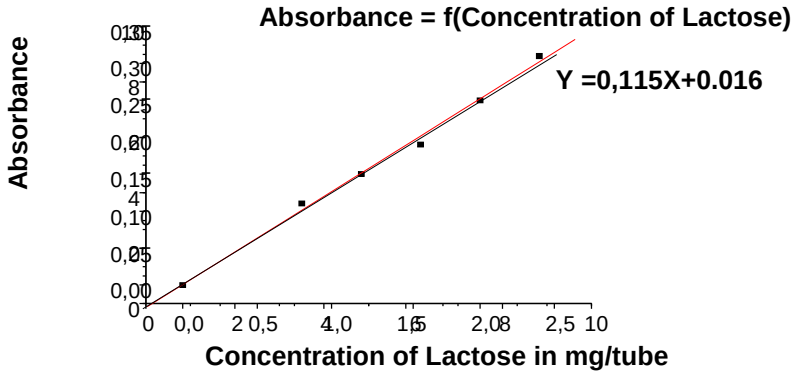

Figure III.16. Curve of lactose content determination

Calculation and reporting: $x = \frac{y-0.016}{0.115}$**;** On the curve, we had C=$\frac{0.154-0.016}{0.115}$= 1.2mg/tube. So, 1.2mg/tube x25=**3.0% or 30g/L.**

The lactose content of raw cow's milk was **48g/L**.

- **Lipids**

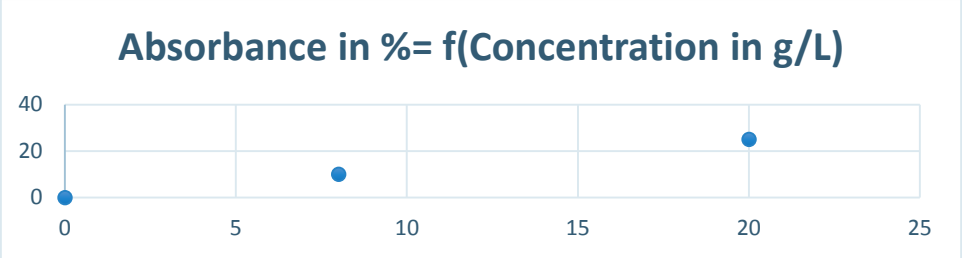

Figure III.17. Curve of lipids content determination

Lipid concentration = (OD dosage/OD standard) x 8g/L= (0.25/0.1)x8= **20g/L**.

The lipid content of raw cow's milk is 37g/L.

- **Proteins**

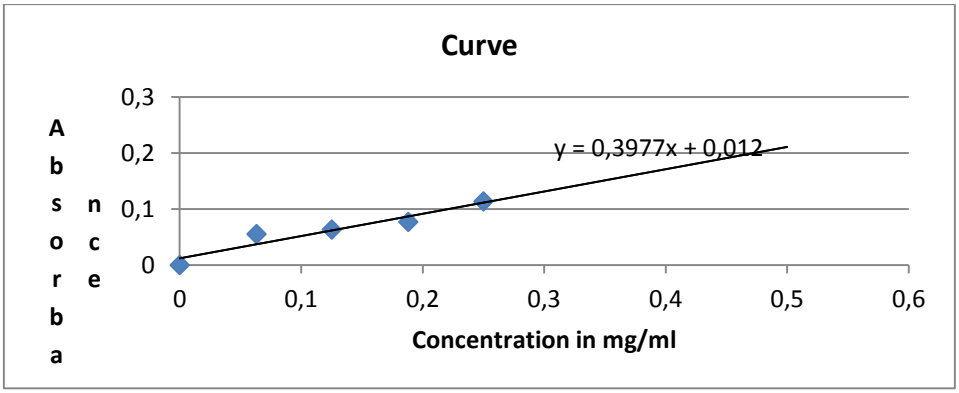

Figure III.18. Curve of proteins content determination

Calculation and reporting: $x = \frac{y-0.012}{0.397}$

On the curve, we have C=$\frac{0.1311-0.012}{0.397}$= 0,30mg/ml. So, 0.30mg/ml x100=**30g/L or 3%.**

- **Minerals**

A porcelain bucket was weighed and the mass obtained of the tare of the crucible was 16.85 g (t). A raw milk mass of 5.30 g (p) was added to the bucket mass and the mass obtained was 22.15 g (a). After 5 hours at 500°C in the oven, we obtained the weight of the crucible and the ash after calcination 16.86 g was (w). After calculation according to the formula (w-t)/(p).100, the ash content was **0.189%** or **1.89g/L**. The criteria of minerals content of raw cow's milk is 9g/L. The minerals content of our raw milk was higher than the criteria.

- **Vitamins A and C characterization**

- **Vitamin A**

The presence of vitamin A was manifested by the appearance of a purple ring on contact with the chloroform solution saturated with antimony. If the medium has been completely dried out, the color changes to bright blue.

- **Vitamin C**

- 5 mL of potassium permanganate solution were taken using a graduated cylinder; they were poured into 3 test tubes, one of which would serve as a control.
- In the first test tube, orange juice was poured using the dropper and count the drops until the potassium permanganate solution was discolored.
- In the second test tube, fresh cow's milk was poured using the dropper and count the drops until the potassium permanganate solution was discolored.
- The 3 three tubes were compared: orange juice, raw cow's milk and control tube.
- We conclude on the vitamin C content of orange juice and raw cow's milk.

III.1.4.4. Microbiological analysis

Microbiological analysis	**Results**	**Criteria**
Microscopic examinations :	**-**	**Absence/25g**
Brucella (Gram Stain)	**Probably presence**	**Absence/25g**
Mycoibacterium (Ziehl Neelsen Stain)	**Probably presence**	**Absence/25g**
Mesophilic aerobic germs (/ml)	**$5.5x10^7$**	**10^6**
Total Coliforms (/ml)	**$1.1\ 10^5$**	**10^2**
Indologenic and sulfhydrogenic bacteria (/L)	**10^6**	**10^2**

Table III.25. Microbiological analysis

- **Microscopic examinations**

- ***Brucella* (Gram Stain)**

The results of the oil immersion staining procedure were observed, examined under a microscope, objective x100.

In the end, Gram negative bacteria would stain pink/red and Gram positive bacteria would stain blue/purple. *Brucella* bacteria were Gram negative.

- ***Mycobacterium* (Ziehl Neelsen Stain)**

We washed and let dry.

We dried and observed by immersion under a microscope, x100 objective.

AARB appeared pink while other microorganisms were blue.

- **Mesophilic aerobic germs (/ml)**

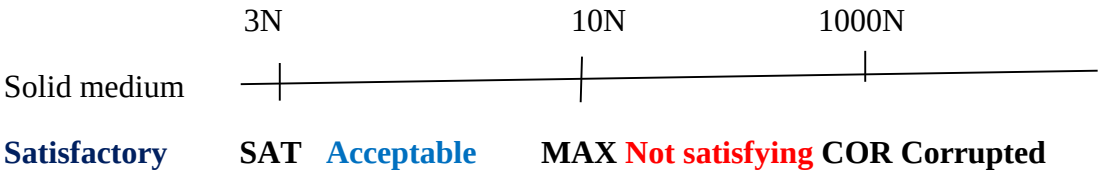

Figure III.19. Criteria scale for MAGs

Dilution	10^{-4}		10^{-5}		10^{-6}	
CFU/Petri dish	Unaccountable	Unaccountable	62	47	12	8

Table III.26. Results of MAGs counting

$$N = \frac{\sum C}{(n1 + 0{,}1n2)V.d}$$

$\sum C = 62 + 47 = 109; n1 = 2 ; n2 = 0 ; V = 1ml$; d= 10^{-5}; N=109 / (2+0.1x0) x1x10^{-5} x 10= **5.5x10^{7} CFU/ml.** The criteria is 10^{6} CFU/ml. The criteria is 55 times (5.5x10^{7}/10^{6}) lower than the results of Mesophilic aerobic germs (MAGs) in our raw milk. So, our raw milk was not satisfying regarding MAGs.

- **Total Coliforms**

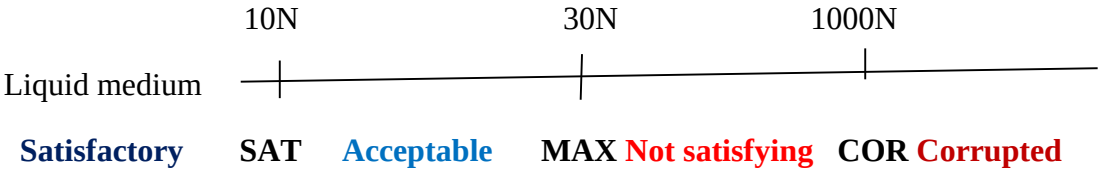

Figure III.20. Criteria scale for total coliforms

Dilution	10^{-2}			10^{-3}			10^{-4}		
Results in tubes	+	+	+	+	+	+	+	+	-
Total positive tubes	3			3			2		

Table III.27. Results of total coliforms counting

All the 6 tubes from dilution 10^{-2} to 10^{-3} were positive and only two tubes were positive at the dilution 10^{-4}. That corresponded to 332, ie 110 on the MacGrady's table. Therefore, we had $110.10^2.10$ = **$1.1x10^5$ Total Coliforms/ml**. the criteria is 10^2 germs /ml. That meaned, we had 1100 times higher than the criterion. So, our raw milk was corrupted regarding « total coliforms ».

- **Indologenic and sulfhydrogenic bacteria**

Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
H_2S (S)	+	+	+	+	+	+
Indole (I)	+	+	+	+	+	+

Table III.28. Results of indologenic and sulfhydrogenic bacteria counting

I = 6 positive tubes; S = 6 positive tubes; I + S = 12.

So, Index (I + S) > 10: our raw milk was withdrawal for legal reason "contamination by numerous microbial germs". **$N \geq 10^6$ germs/ml.** It was 10,000 times ($10^6/10^2$) higher than the criteria.

III.1.5. Results of the year 2022

Analysis and Criteria	Year 2022			
	T1	**T2**	**T3**	**T4**
Organoleptic Analysis				
Temperature (°C)	**35**	**34.5**	**35.5**	**33**
Appearance and texture : **Opaque to light**	**Opaque to light**	**Opaque to light**	**Opaque to light**	**Opaque to light**
pH : **6.7 +/- 0.1**	**6.40**	**6.31**	**6.33**	**6.40**
Dornic acidity **(°D)** : **16 - 18**	**27**	**31**	**30**	**25**
Color : **Pearl white**	**Pearl white**	**Pearl white**	**Pearl white**	**Pearl white**
Odor : **related to fats**	**+**	**+**	**+**	**+**
Density : **1.032 – 1.035 at 25°C**	**1.02 at 25°C**	**1.13 at 25°C**	**1.02 at 25°C**	**1.03 at 25°C**
Conclusion	**Unsatisfac-tory organoleptic quality**	**Unsatisfac-tory organoleptic quality**	**Unsatis-factory organoleptic quality**	**Unsatisfactory organoleptic quality**
Nutritional Analysis				
Water (g/L) : **820 - 890**	**856.9**	**836.9**	**829.1**	**843.2**
Lactose (g/L) : **48**	**20**	**28**	**32**	**30**
Lipids (g/L) : **37**	**20**	**21**	**23**	**20**
Proteins (g/L) : **34**	**30**	**28**	**30**	**30**
Minerals (g/L) : **9**	**1.85**	**3.38**	**3.82**	**1.89**
Vitamins A and C characterization : **1**	**A+ C+**	**A+ C+**	**A+ C+**	**A+ C+**
Conclusion	**Unsatisfacto-ry nutritional quality**	**Unsatisfacto-ry nutritional quality**	**Unsatis-factory nutritional quality**	**Unsatisfactory nutritional quality**
Microbiological Analysis				
Microscopic examinations : **Absence**	**Absence**	**Absence**	**Absence**	**Absence**
Brucella (Gram Stain)	**Presence**	**Presence**	**Presence**	**Presence**
Mycobacterium (Ziehl Neelsen Stain)	**Presence**	**Presence**	**Presence**	**Presence**
Mesophilic aerobic germs (/ml) : 10^6	$4.6x10^7$	$5.2x10^7$	$7.0x10^7$	$5.5x10^7$
Total Coliforms (/ml) : 10^2	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$
Indologenic and sulfhydrogenic bacteria (/ml) : 10^2	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$
Conclusion	**Very poor microbiologi-cal quality**	**Very poor microbiologi-cal quality**	**Very poor microbiologi cal quality**	**Very poor microbiological quality**

Table III.29. Analysis summary

III.1.5.1. First trimester (T1)

- **Organoleptic Analysis**

With the exception of appearance, texture, color and odor, the other results (pH, Dornic acidity and density) didn't meet the criteria. Our raw milk therefore didn't meet all the organoleptic criteria according the Codex Alimentarius. So, it had an unsatisfactory organoleptic quality. Even if the viscosity had been determined, our raw milk would not fulfill all the conditions of satisfaction.

- **Nutritional Analysis**

Except the amount of water and the present of vitamins A and C, the other results (lactose, lipids, proteins and minerals) didn't meet the criteria. Our raw milk therefore didn't meet all the nutritional criteria according the Codex Alimentarius. So, it had an unsatisfactory nutritional quality.

- **Microbiological Analysis**

No result met the microbiological criteria. *Brucella* and *Mycobacteria* (AARBs) were suspected to be present rather than absent.

Mesophilic aerobic germs, total coliforms and indologenic and sulfhydrogenic bacteria were present in our raw milk in unsatisfactory quantities. In food microbiology, at least two (2) unsatisfactory results give a corrupted product. So, our raw milk had a very poor microbiological quality.

III.1.5.2. Second trimester (T2)

- **Organoleptic Analysis**

Like the first quarter results, only appearance, texture, color and odor meet the criteria. Our raw milk therefore didn't meet all the organoleptic criteria according the Codex Alimentarius. So, it had an unsatisfactory organoleptic quality.

- **Nutritional Analysis**

Except the amount of water and presence of vitamins A and C, the other results (lactose, lipids, proteins and minerals) didn't meet the criteria. Our raw milk therefore didn't meet all the nutritional criteria according the Codex Alimentarius. So, it had an unsatisfactory nutritional quality.

- **Microbiological Analysis**

No result met the microbiological criteria. *Brucella* and *Mycobacteria* (AARBs) were suspected to be present rather than absent.

Mesophilic aerobic germs, total coliforms and indologenic and sulfhydrogenic bacteria were present in our raw milk in unsatisfactory quantities. In food microbiology, at least two (2) unsatisfactory results give a corrupted product. So, our raw milk had a very poor microbiological quality.

III.1.5.3. Third trimester (T3)

- **Organoleptic Analysis**

As in previous quarters, only appearance, texture, color and odor meet the criteria. Our raw milk therefore didn't meet all the organoleptic criteria according the Codex Alimentarius. So, it had an unsatisfactory organoleptic quality.

- **Nutritional Analysis**

Except the amount of water, the other results (lactose, lipids, proteins, minerals and vitamins A and C) didn't meet the criteria. Our raw milk therefore didn't meet all the nutritional criteria according the Codex Alimentarius. So, it had an unsatisfactory nutritional quality.

- **Microbiological Analysis**

No result met the microbiological criteria. *Brucella* and *Mycobacteria* (AARBs) were suspected to be present rather than absent.

Mesophilic aerobic germs, total coliforms and indologenic and sulfhydrogenic bacteria were present in our raw milk in unsatisfactory quantities. In food microbiology, at least two (2) unsatisfactory results give a corrupted product. So, our raw milk had a very poor microbiological quality.

III.1.5.4. Fourth trimester (T4)

- **Organoleptic Analysis**

With the exception of appearance, texture and color, the other results (pH, Dornic acidity, odor and density) didn't meet the criteria. Our raw milk therefore didn't meet all the organoleptic criteria according the Codex Alimentarius. So, it had an unsatisfactory organoleptic quality.

- **Nutritional Analysis**

Except the amount of water, the other results (lactose, lipids, proteins, minerals and vitamins A and C) didn't meet the criteria. Our raw milk therefore didn't meet all the nutritional criteria according the Codex Alimentarius. So, it had an unsatisfactory nutritional quality.

- **Microbiological Analysis**

No result met the microbiological criteria. *Brucella* and *Mycobacteria* (AARBs) were suspected to be present rather than absent.

Mesophilic aerobic germs, total coliforms and indologenic and sulfhydrogenic bacteria were present in our raw milk in unsatisfactory quantities. In addition, indologenic and sulhydrogenic bacteria were withdrawal for legal reason "contamination by numerous microbial germs" during all quarters.

In food microbiology, at least two (2) unsatisfactory results give a corrupted product. So, our raw milk had a very poor microbiological quality.

By the way, the *Codex Alimentarius* has several requirements related to milk to be good for human health, such as [**6]:**

- Milk is the normal mammary secretion of milking animals obtained from one or more milkings without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing ;
- Milk which is modified in composition by the addition and/or withdrawal of milk constituents may be identified with a name using the term "milk", provided that a clear description of the modification to which the milk has been subjected is given in close proximity to the name.

We can say that our raw milk didn't meet all the organoleptic, nutritional and microbiological criteria to be consumed without prior technological treatment.

However, we will question the veracity of our results. We were therefore going to open a discussion in order to better understand the real origins and parameters of the non-compliance of our raw cow's milk for human consumption.

III.2. DISCUSSION

The physico-chemical and organoleptic analysis shows that the results seem to be strongly influenced mainly by the conditions of milking (health of the cows, handlers, etc.) and sale of the raw milk and secondarily by the temperature, the pH, the chemical composition milk.

The values of the different samples taken have approximately the same physico-chemical characteristics from one season to another. This resemblance in the values of the physico-chemical parameters is linked to the characteristics of the seasons which are quite similar despite the period of rain and drought.

Temperature is also an important environmental factor for the life of microorganisms. It thus controls all the biological processes (reproduction, growth, etc.) linked to a given environment. The temperatures recorded during the samplings are between 30°C and 35°C. This interval would favor the proliferation of most microorganisms **[1] [7].**

Finally, none of these hypotheses is able to individually explain the appearance of an inclusive category of microorganisms and these hypotheses are not mutually exclusive. However, no factor taken individually is able to predict or explain the cause of the dominance of certain microorganisms over others due to poor hygienic conditions **[1] [7] [22] [23].**

According to **[22]**, milk which is already rich in nutrients, even in small quantities at temperatures between 15 and 30°C and with a pH varying from 6 to 9, favors the proliferation of most microorganisms.

Indeed, all this can promote the multiplication and growth of most toxigenic and pathogenic microorganisms.

Moreover, microorganisms are present in all foods. But microbial populations do not stay fixed. They evolve according to **[1] [7]**:

- Nutrients (sources of energy: carbohydrates above all, lipids possibly, sources of assimilable nitrogen, necessary for the production of proteins and genetic factors: amino acids, simple peptides, growth factors: vitamins, certain minerals).
- Water is both a carrier of germs and an essential element for life. It is fundamental for the multiplication of germs and the germination of spores.
- Temperature: Different categories of microbes with temperature requirements for growth are: psychrophilic (≤0°C), psychrotrophs (4 to 25°C), mesophile (20 to 45°C), thermophile (50 to 80°C), hyperthermophile (80 to 110°C).

- pH: The ranges used are for molds: pH between 1.5-2 and 11; optimum: 7; for yeasts: pH between 2.5 and 8.5; optimum: 6.5 and for bacteria: pH between 3.8 and 9; optimum: 7.
- Redox potential, etc.

The microbiological analysis revealed the presence of all the germs sought in excess (*Brucella* and *Mycobacterium* seen under microscope, mesophilic aerobic, total coliforms and indologenic and putrid bacteria). This may be due to the influence of several parameters such as: Raw material, the environment, labor (Men), material, methods that are very good conductors of microorganisms **[1] [7].**

- Raw material
  - ✓ The cow itself, if it is sick, can excrete *Brucella, Mycobacterium* in the milk;
- The milieu (environment)
  - ✓ Soil and plants almost all bring germs, in very large quantities.
  - ✓ Natural, untreated water, from a source or from a river, also brings many germs, which often come from the soil. However, we can also find pollution germs, resulting from runoff: *Enterobacteria* (from animal feces), *Clostridium*, including certain pathogenic germs such as *Salmonella typhi*.
  - ✓ Air and dust are mainly transport agents. They are essentially vectors of exogenous spores and bacteria.
- Men : Humans are a very contaminating agent by:
  - ✓ Their hands: the skin contains permanent germs, such as certain Staphylococci, and often pathogenic transition germs (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas*, etc.). The hands can carry *Enterobacteriaceae* of fecal origin.
  - ✓ Their nasal or oral secretions may contain *Staphylococci.*
  - ✓ Their dust-bearing clothes are therefore carriers of mold and spores.
- Material: Equipment and instruments carry germs from contaminated water or other food in the event of poor cleaning and disinfection practices.
- Methods : Milking methods can be a disorganization of production by:
  - ✓ Cross contamination;
  - ✓ A loss of risk control.

GENERAL CONCLUSION

The abundance of microorganisms would be related to the milking conditions during all seasons (rainy season and dry season) and they proliferate both in the dry season and in the rainy season.

The organoleptic parameters that allowed microorganisms to contaminate raw cow's milk are:

- The temperature of the milk: their optimum growth (microorganisms) is, for the most part, at temperatures above 30°C;
- pH and Dornic acidity: microorganisms, which can develop at pH above 6.3;
- The opacity could be due to elements added to the milk to increase density and viscosity to give the impression of good raw milk. This can be ruled out by nutritional analysis. However, the other parameters: appearance and texture, color, smell, remained intact or close to the characteristics of raw cow's milk.

Nutrients didn't meet the criteria. We have:

- Water: Although meeting the criteria (82-89%), it had to be hidden or bound by adding other elements such as: starch, fat removal.
- Lactose: The assay revealed a lactose level of 20 to 30 g/L, below the criterion (48g/L). However, the quantity is not negligible. The value had to be reduced during the fat removal that milkers sell differently. There is also the presence of lactic bacteria which influenced the pH (6.31 to 6.40) and the Dornic acidity (25 to 30°D) by breaking down lactose into lactic acid.
- Lipids: The amount was between 20 and 23g/L. They do not meet the criteria (37g/L), it had to be hidden or bound by adding other elements such as: starch, fat removal. Lipolytic bacteria are also a factor in lowering the quantity of lipids in Milk.
- Proteins: The amount was between 28 and 30g/L. They did not meet the criteria (34g/L), it had to be hidden or bound by adding other elements such as: starch, fat removal. Proteolytic bacteria (sulfohydrogens and indologens) also reduced the amount of protein there.
- Minerals: They were low (1.85 to 3.82g/L). They did not meet the criteria (9g/L);
- Vitamins A and C (the criteria is 1g/L): They were qualitatively present even if it is in very small quantity. We are glad that the trading conditions did not destroy them. Minerals and vitamins are growth factors for microorganisms, hence their low levels.

The microbiological analysis revealed that the raw cow's milk from the Bagnon market has a very poor microbiological quality throughout the year despite the sampling done at different sampling points. No microorganism met the microbiological criterion according to the *Codex Alimentarius*. It was:

- General hygiene test microorganisms including :
  - ✓ Microorganisms affecting marketable quality: for example mesophilic aerobic germs;
  - ✓ Index or indicator microorganisms: Coliforms, Indologenic and putrid bacteria;
- Potentially pathogenic microorganisms: *Brucella* and *Mycobacteria* for example.

According to the Codex alimentarius, a Good-quality raw cow's milk has to be free of debris and sediment, free of off-flavors and abnormal color and odor; low in bacterial count; free of chemicals (e.g., antibiotics, detergents); abnormal acidity but it must have a normal nutritional composition.

Our raw milk is certainly free of debris and sediment, free of off-flavors and abnormal color and odor. However, it had not a good quality because it met no nutritional and microbiological quality. The excessive presence of microorganisms (which can cause microbial food-borne illnesses) in addition, significantly reducing the nutrient content.

Local production of milk is an objective in order to reduce import costs. So, the quality of raw milk is the primary factor determining the quality of milk products. Good-quality milk products can be produced only from good-quality raw milk (according to the *Codex alimentarius*). In addition, the study allowed us to understand that there can be physical, chemical, allergic but above all microbiological dangers. So, the focus should be more on hygienic quality.

The hygienic quality of milk is of crucial importance in producing milk and milk products that are safe and suitable for their intended uses.

To achieve this quality, good hygiene practices should be applied throughout the dairy chain. Among the causes of small-scale dairy producers' difficulties in producing hygienic products are informal and unregulated marketing, handling and processing of dairy products; lack of financial incentives for quality improvement, and insufficient knowledge and skills in hygienic practices. However, we would like to propose some hygiene methods in order to improve the hygienic quality of milking in the table below:

5Ms of Quality	Difficulties, dangers or risks	Solutions
Raw Material	• The cow itself, if it is sick, can excrete *Brucella*, *Mycobacterium* in the milk	The dairy cow itself must be in good health.
Milieu (Environment)	• Soil and plants almost all bring germs, in very large quantities. • Natural, untreated water, from a source or from a river, also brings many germs, • Air and dust are mainly transport agents. They are essentially vectors of exogenous spores and bacteria.	Protect dairy milking contamination from dust, leaves, water, air, etc.
Men	Humans are a very contaminating agent by: • Their hands: the skin contains permanent germs, such as certain Staphylococci, and often pathogenic transition germs. • Their nasal or oral secretions may contain *Staphylococci.* • Their dust-bearing clothes are therefore carriers of mold and spores. • Lack of competence, communication, bad training	Respect body and clothing hygiene by being trained in good hygiene practices and by wearing clean clothes, headgear, mufflers, etc.
Material	• Equipment and instruments carry germs from contaminated water or other food in the event of poor cleaning and disinfection practices.	Work in good hygienic conditions, with adequate and disinfected equipment.
Methods	• Cross contamination; • A loss of risk control	Avoid any risk of contamination by working with hygienically appropriate methods.

Table III.30. 5 Ms of Quality

The study therefore allowed us to review the methods of hygiene, cleaning, disinfection, milking, and the HACCP system.

For example, the study of the 5 Ms showed us that cleaning and disinfection were not carried out well. As for the importance and implementation of the HACCP system, milkers (mostly illiterate) have not yet understood them, certainly through negligence, ignorance or bad faith.

Indeed, we would like to make practical proposals for the organization of the raw milk sector:

- Propose adequate infrastructure with drinking water points and toilets as well as appropriate equipment;
- Put in place a national food hygiene policy;
- Put in place health measures, i.e. the establishment of an annual health card at a reduced rate for handlers of raw milk and issued by an authorized doctor (medical monitoring); the best can be awarded;
- Put in place hygiene measures: ensure the safety (cleaning of spaces and sewers, collection of household waste, etc.) of places of activity by the public authorities (Town Hall, District, Government).

To attract everyone's attention, we are therefore going to give all the probable poisonings due to the consumption of raw cow's milk, of course, but also to the consumption of any food product that can cause food poisoning; especially since certain strands of food (cereals, legumes, meat, etc.) can be found in the milk during milking and sale.

- Establish a framework of good hygienic practice;
- Establish a code of practice in the raw milk sector;
- Inform, train and educate economic operators in food and health hygiene:
- Make certain economic operators literate;
- Initiate and organize information forums in the municipalities for those concerned;
- Facilitate public awareness campaigns in this area;
- Do a routine check.

Furthermore, for the effective and efficient application of our proposals, we rely heavily on the power of the following personalities:

- Elected officials who must fully assume their responsibilities: imposing or adopting basic legislative texts but also modernizing slaughterhouses, markets and collective catering as quickly as possible;

- Teachers who must make all students aware of these everyday perils, explain to them the interest and the rules of hygiene and then get them used to observing before choosing the cleanest suppliers;
- Journalists who finally have an essential mission in this area, because they can complete the education of all consumers.

However, our work is subject to constructive criticism in order to improve the hygienic quality of our raw cow's milk in the interest of all.

Finally, as recommendations, we suggest for the continuation of the research, that the student researcher concerned emphasizes the positive impact of our study in the raw dairy sector by:

- Verification of the effective application of food hygiene;
- Verification of the effectiveness of the cleaning and disinfection procedure using appropriate methods (microbiological for example);
- Verification of the implementation of the HACCP system, the 5 Ms;
- Frequent microbiological analysis of raw milk to get an exact idea of the proliferation of microorganisms in raw milk and the type of microorganisms responsible for collective food poisoning;
- Complete analysis (biological, physical, chemical) of raw milk;
- If possible and necessary, carry out in-depth analyzes by PCR, HPLC, GC, etc.

ACKNOWLEDGMENTS

- All our thanks go to the staff and all the trainers of the Polytechnic Doctoral school of the Houphouet Boigny- National Polytechnic Institute (Yamoussoukro) and Selinus University (Italy), specially:
- Dr. Salvatore Fava, UNISE (Our General Supervisor) and his collaborators for their efforts in order to succeed our PhD Thesis in Food Sciences ;
- Professor Kouassi Benjamin Yao, Director of the Polytechnic Doctoral School (EDP) of the Houphouet Boigny - National Polytechnic Institute (INP-HB Yamoussoukro, Côte d'Ivoire), for accepting us as a student in his school;
- Doctor Aw Sadat, Lecturer at the EDP, INP-HB (Yamoussoukro), who has prepared to tackle doctoral thesis research work;
- Doctor Aw Ngoran Bla Zita, Lecturer at the EDP, INP-HB (Yamoussoukro), who allowed us to enroll in a doctoral school;
- Doctor Koutouan Desire Abro, Department Assistant at the EDP, INP-HB (Yamoussoukro), for his collaboration ;
- Doctor Doudjo Soro, Director of studies at the EDP, INP-HB (Yamoussoukro), for his collaboration ;

Ladies:

- Ouara, Spouse Kouakou Akissi, Manager of the Higher Technical School of Yopougon for her encouragement in training;
- Gisele Sanger, secretary of the EDP, INP-HB (Yamoussoukro).

We also thank:

- All the shepherds of the Pasteur Institute of Côte d'Ivoire (Adiopodoume) and the sellers of raw cow's milk at the Bagnon market (Yopougon, Abidjan) for their availability to answer our questions;
- Mrs. Rose KOUAO, documentlist at the FAO for her availability and sympathy;
- And all those who directly or indirectly contributed to the development of this Ph.D. Thesis.

Finally, we would like to thank all the people who were kind enough to answer our questions and allow us to carry out our research work.

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31) **République Française** (1997), Hygiène en restauration collective, Arrêté du 29 septembre 1997 fixant les conditions d'hygiène applicables dans les établissements de restauration collective à caractère social, Paris, 16 pages.

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INTERNET RESOURCES

33) **Nutritional benefits of fat soluble vitamins**

34) **Nutritional benefits of water soluble vitamins**

ANNEXES

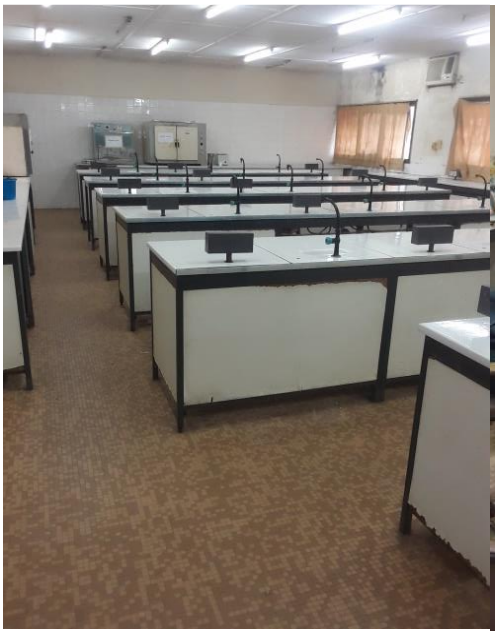
ANNEX A1: Milking and sale of raw cow's milk

Milking raw cow's milk

Different points of sale for raw cow's milk (Bagnon market)

ANNEX A2: Laboratories


Laboratory of Biochemistry and Reserves




Laboratory of Microbiology and Reserves

ANNEX A3: Reagents used for the determination of nutrients in fresh milk

1) **Lactose determination**
   - **DNS**

I prepared DNS reagent using the following steps:

- Dissolve 1g of 3,5 dinitrosallicylic acid in 20mL of 2M NaOH.
- Then add slowly 30g sodium potassium tartrate and dilute to a final volume of 100mL using distilled water.

2) **Lipids determination**
   - **SPV reagent**

- A phospho-vanillin reagent was prepared by dissolving 6 grams vanillin in water in a 1 liter flask and diluting to volume with water.
- 350 ml. of this vanillin reagent was admixed with 50 ml. of water in a 2 liter flask.
- Thereafter, 600 ml. of concentrated phosphoric acid, specific gravity 1.7, was added to the vanillin reagent with constant stirring.
- The mole ratio of vanillin to phosphoric acid in the reagent is 1.56 × 10- 3.

3) **Proteins determination**
   - **Folin-Ciocalteu´s phenol reagent**

- Dissolve 10 g of sodium tungstate and 2.5 g of sodium molybdate in 70 ml of water.
- Add 5 ml of 85% phosphoric acid and 10 ml of concentrated hydrochloric acid.
- Reflux for 10 hours.
- Add 15 g of lithium sulfate, 5 ml of water, and 1 drop of bromine. Reflux for 15 minutes.
- Cool to room temperature and bring to 100 ml with water.

ANNEX A4: Diluent, Culture Media (g /Liter) and Reagent preparation

1) Buffered Peptone Water

- Enzymatic digest of casein: 10.0
- Sodium chloride: 5.0
- Disodium hydrogen phosphate (anhydrous): 3.5
- Potassium dihydrogen phosphate: 1.5

pH 7.0 ± 0.2 at 25°C

- Add 20.0g of Buffered Peptone Water (ISO) to 1 liter of distilled water.
- Mix well and distribute into final containers. Sterilize by autoclaving at 121°C for 15 minutes.

2) MacConkey broth

- Peptone : 20
- Lactose monohydrate: 10
- Bile salts: 5
- Sodium chloride : 5
- Bromocresol purple: 0.01

pH 7.4 ± 0.2 at 25°C

- To prepare single strength broth, add 40g to 1 liter of distilled water.
- Distribute into containers fitted with fermentation (Durham) tubes.
- Sterilize by autoclaving at 121°C for 15 minutes.

3) Nutrient Agar

- Agar : 15
- Peptone: 5.0
- Yeast extract: 3
- NaCl : 5

pH 7.4 ± 0.2 at 25°C

- Suspend 28 grams in 1000 ml distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
- Cool to 45-50°C.

4) Peptone water cysteine with iron citrate

- Peptone : 20
- NaCl : 5
- Cysteine : 0,2
- Ammonia iron citrate : 0.5

- After the addition of all ingredients, sterilize by autoclaving at 121 °C for 15 min.
  Final pH: 6.0–6.5.

5) Kovac's reagent: Formulation per 100 mL

p-Dimethylaminobenzaldehyde : 5 g ; Isoamyl Alcohol : 75mL; Hydrochloric Acid : 25 mL

-Using a sterile inoculating loop, lightly inoculate Tryptone Broth using growth from an overnight, pure culture plate.
-Incubate at 35°C for 24 or 48 hours. If testing is performed after 24 hours it is recommended that a 2.0-mL portion be removed aseptically for the test. If negative, the remaining broth should be reincubated for an additional 24 hours and retested.
-Add five drops (0.5-mL) of Kovac's Reagent and shake the tube gently.
-Check for a color change immediately.
The active ingredient in Kovacs Reagent, p-dimethylaminobenzaldehyde, reacts with indole to form a pinkish-red end product that is highly visible.

ANNEX A5: Preparation of reagents (Gram and Ziehl Neelsen Stain)

1) Gram Stain

- **Crystal violet**

- Dissolve 2 g certified crystal violet into 20 ml of 95% ethyl alcohol.
- Dissolve 0.8 g ammonium oxalate into 80 ml distilled water.
- Mix the two solutions together and allow them to stand overnifght at room temperature (25°C).
- Filter through coarse filter paper before use.
- Store at room temperature (25°C).

- **Gram's iodine**

- Grind 1.0 g iodine (crystalline) and 2.0 g potassium iodide in a mortar. Small additions of distilled water may be helpful in preparing the solution.
- Add to 300.0 ml distilled water.
- Store at room temperature (25°C) in a foil-covered bottle (to protect solution from light).

- **Decolorizer**

Some workers prefer to use acetone by itself, ethanol 95% v/v, or ethanol-iodine as the decolorizing solution. A mixture of acetone-alcohol is recommended because it decolorizes more rapidly than ethanol 95% v/v, and is less likely to over-decolorize smears than acetone without alochol added.

- **Safranin**

- Add 2.5 g certified safranin-O to 100.0 ml 95% ethyl alcohol.
- Add 10.0 ml safranin and ethyl alcohol solution made in step 1 to 90.0 ml distilled water.
- Store at room temperature (25°C).

2) Ziehl Neelsen Staining

- **Carbol fuschin**

- Distilled water- 100ml
- Basic fuschin- 1g
- Ethyl alcohol (100% ethanol)- 10ml
- Phenol crystals- 5ml

- **0.25% methylene blue in 1% acetic acid**

- Methylene blue- 0.25g
- Distilled water- 99ml
- Acetic acid- 1ml

- **Acid alcohol (3% hydrochloric acid in 95% ethyl alcohol)**

- Ethyl alcohol- 95 ml
- Distilled water- 2 ml
- Concentrated hydrochloric acid- 3 ml

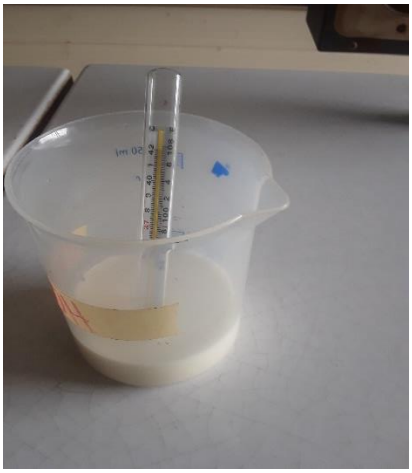
ANNEX A6: Mac Grady's Table

Tables NPP (d'après la norme ISO 7218 :1996(F))

Tableau 1 - Table NPP pour 3 x 1 g (ml), 3 x 0,1 g (ml) et 3 x 0,01 g (ml).

Nombre de résultats positifs			NPP	Catégorie lorsque le nombre d'essais de mesures est de 1 pour le lot considéré	Limites de confiance			
					>95%	>95%	>99%	>99%
0	0	0	<0,30		0,00	0,94	0,00	1,40
0	0	0	0,30	3	0,01	0,95	0,00	1,40
0	1	0	0,30	2	0,01	1,00	0,00	1,60
0	1	1	0,61	0	0,12	1,70	0,05	2,50
0	2	0	0,62	3	0,12	1,70	0,05	2,50
0	3	0	0,94	0	0,35	3,50	0,18	4,60
1	0	0	0,36	1	0,02	1.70	0,01	2,50
1	0	1	0,72	2	0,12	1,70	0,05	2,50
1	0	2	1,1	0	0,4	3,5	0,2	4,6
1	1	0	0,74	1	0,13	2,00	0,06	2,70
1	1	1	1,1	3	0,4	3,5	0,2	4,6
1	2	0	1,1	2	0,4	3,6	0,2	4,6
1	2	1	1,5	3	0,5	3.8	0,2	5,2
1	3	0	1,6	3	0,5	3,8	0,2	5,2
2	0	0	0,92	1	0,15	3,50	0,07	4,60
2	0	1	1,4	2	0,4	3,5	0,2	4,6
2	0	2	2	0	0,5	3,8	0,2	5,2
2	1	0	1,5	1	0,4	3,8	0,2	5,2
2	1	1	2,0	2	0,5	3,8	0.2	5,2
2	1	2	2,7	0	0,9	9,4	0,5	14,2
2	2	0	2,1	1	0,5	4,0	0,2	5,6
2	2	1	2,8	3	0,9	9,4	0,5	14,2
2	2	2	3,5	0	0,9	9,4	0,5	14,2
2	3	0	2,9	3	0,9	9,4	0,5	14,2
2	3	1	3,6	0	0,9	9,4	0,5	14,2
3	0	0	2,3	1	0,5	9,4	0,3	14,2
3	0	1	3,8	1	0,9	10,4	0,5	15,7
3	0	2	6,4	3	1,6	18,1	1,0	25,0
3	1	0	4,3	1	0,9	18,1	0,5	25,0
3	1	1	7,5	1	1,7	19,9	1,1	27,0
3	1	2	12	3	3	36	2	44
3	1	3	16	0	3	38	2	52
3	2	0	9,3	1	1,8	36,0	1,2	43,0
3	2	1	15	1	3	38	2	52
3	2	2	21	2	3	40	2	56
3	2	3	29	3	9	99	5	152
3	3	0	24	1	44	99	3	152
3	3	1	46	1	9	198	5	283
3	3	2	110	1	20	400	10	570
3	3	3	>110					
autres valeurs			non cité dans la table ISO 7218 : 1996 (F)					

ANNEX 7: Organoleptic characteristics of raw milk



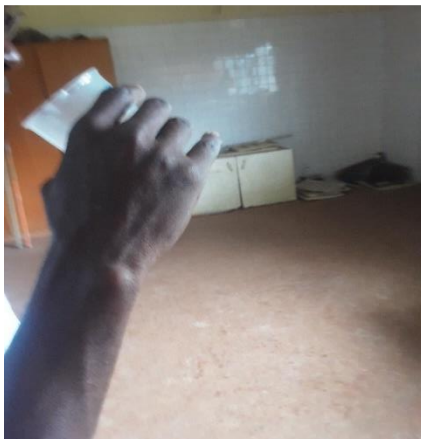
Temperature

Appearance and texture

pH

Dornic acidity

Color



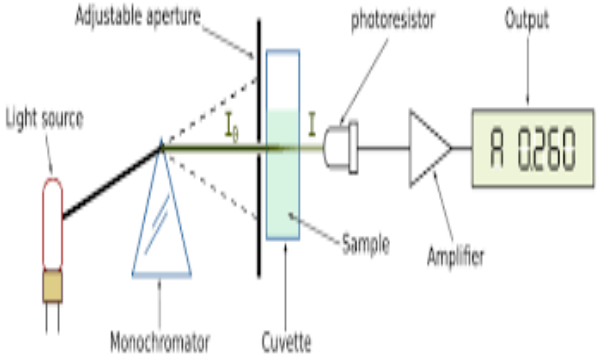
Odor

Density

ANNEX 8: Nutrients in raw milk

Water

Proteins



Principe of Spectrophotometer

Lipids and Lactose determination

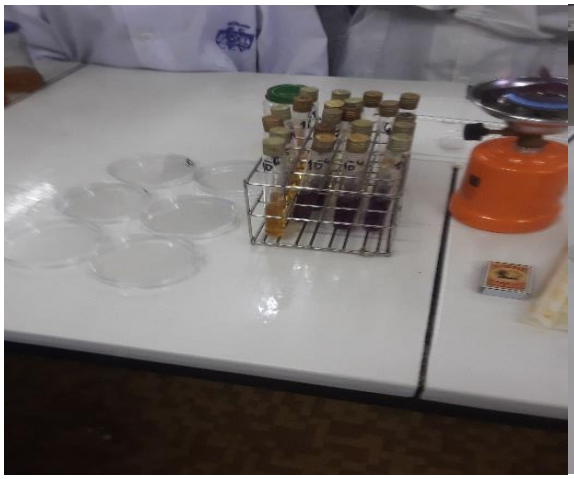
Minerals

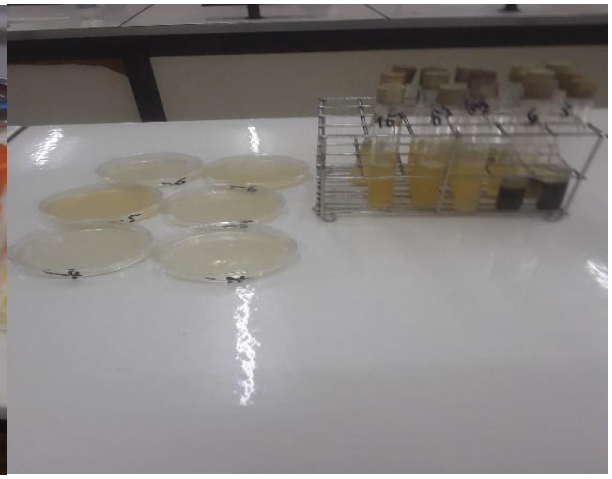
Before

After

Vitamins A and C characterization

ANNEX 9: Microbiological analysis





Before **After (results)**

Microbiological analysis (Mesophilic aerobic germs, Total Coliforms, Indologenic and sulfhydrogenic bacteria)

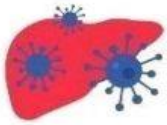
Microscopic examinations: *Brucella* (Gram Stain) and *Mycobacterium* (Ziehl Neelsen Stain)

ANNEX 10: Big 6 Foodborne Illnesses and their Prevention

Big 6 Foodborne Illnesses

Norovirus

- Direct contact with the infected
- Contact with fecal matter
- Bodily fluids transfer to food
- Contaminated water

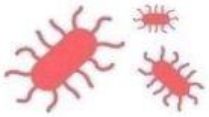


Hepatitis A

- Contaminated food and water
- Contact with fecal matter
- Cross-contamination

E.coli

- Contact with fecal matter
- Contaminated food and water
- Undercooked meat
- Raw milk



Non-typhoidal

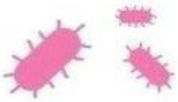
Salmonella

- Food of animal origin (e.g., poultry, eggs and other meat)
- Contaminated fruits and vegetables
- Processed foods (e.g., peanut butter and frozen pies)
- Contaminated water

Typhoidal

Salmonella

- Undercooked meat (e.g., poultry, beef, and pork)
- Contaminated raw fruits and vegetables
- Raw/undercooked eggs and egg products
- Raw milk



Shigella

- Contaminated food and water
- Sick food handler
- Contact with fecal matter
- Cross-contamination

How to prevent?

Always properly wash hands.

Cook foods thoroughly to the correct internal temperature.

Use separate utensils and equipment for raw and ready-to-eat foods.

Store foods separately and in the correct areas and temperature.

Sick workers must avoid attending service.

Use a clean source of food and water.

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ANNEX 11: Food Safety Management System