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Physicochemical and microbiological quality analyses of cow's raw milk raised in a rural region and an urban region.

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Nuseiba

DEDICATION

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Abbreviations list

% :	Percent
°C :	Degree Celsius
°D :	Dornic Degree
Spp :	Standing for species pluralis, the Latin for multiple species
NPN :	Non-protein nitrogen
FGM :	Fat globule membrane
D :	Density
ADSA :	American Dairy Science Association's
pH :	Potential of Hydrogen
e.g. :	Exempli gratia, meaning for example
°F :	Degree Fahrenheit
FAO :	Food and Agriculture Organization
Mml :	Millimoles
µ :	Micro
µmol :	Micromole
i.e. :	Latin phrase id est, meaning that is
G :	Gram
H+ :	Hydrogen ion
V :	Volume
DM :	Dry matter
DDM :	Defatted dry matter
STEC:	Shiga toxin-producing Escherichia coli
L :	Liter
Cm :	Centimeter
MG :	Milligram
PCA :	Plate count agar
H :	Hour
MM :	Millimeter
ML :	Milliliter
BCPL :	Bromo Cresol Purple Lactose
IMI :	Intramammary infections
SCC :	Somatic cell count
BPW :	Buffered peptone water
CFU :	Colony Forming Unit
BSA :	Bovine serum albumin

Introduction

"Raw milk" is a milk produced by the secretion of the mammary gland of farmed animals and not heated to more than 40°C, nor subjected to a treatment of equivalent effect according to the **((EC) No 853/2004).** Raw milk for human consumption must be free of pathogens.

Due to its unique nutritional quality, milk is a complete food that can guarantee a large supply of protein, lipids, mineral, salts, especially calcium, phosphorus and vitamins **(FAO, 2012).** This food occupies an important place in the diet of Algerians, it provides them with the largest source of animal protein, and is considered a key player in the agri-food industry **(Anonymel, 2008).**

Algeria is the largest consumer of dairy products in the Maghreb region. It is estimated that the country's annual output is 2.2 billion liters, including 1.6 billion liters of raw milk per capita consumption in 2010 should reach 115 liters. It is estimated that the average annual growth rate of the Algerian dairy market is 20%.

Milk is an unstable microbial product because it is a favorable growth environment for the proliferation of various microbial flora. In order to ensure good consumer protection, it is necessary to control the hygienic conditions from milking to finished product **(Guiraud, 1998).** Through strict control of the physical and chemical and bacterial quality of milk.

In this context, we carried out this research based on the analysis of the physicochemical and microbiological quality of raw milk collected from Ras El Agba a rural village in Guelma province and a sample from an unauthorized farm that feed cows from garbage containers in Bourwayah district in the city center of Guelma province.

Bibliographic part

Generalities on milk

I. Generalities on milk

I.1. Definition of milk

Milk is an opaque liquid with a matte white color. It is more or less light yellow according to the content of fat and β-carotene, with a weak odor and slightly sweet taste. It is secreted by the mammary glands of female mammals after the birth of their young. According to the international conference on suppression of fraud held in Geneva in 1908: "milk is a part of a healthy, well-nourished and non-overworked lactating mother's comprehensive uninterrupted milking. It must be collected correctly and must not contain colostrum." **(Alais, 1975).**

The Codex Alimentarius in 1999 defines it as the normal mammary secretions of milking animals obtained from one or more milkings, without addition or extraction, and intended for consumption or further processing as liquid milk.

Jeantet *et al.*, (2008) report that milk must also be collected under good hygienic conditions and provide all health guarantees. It can be sold as is, but the most common is lipid standardization and microbial purification to limit hygiene risks and ensure longer storage.

Milk is a very complex mixture consisting of fat in the form of an emulsion, protein in the form of a colloidal suspension, sugar and salt in the form of a solution. In addition, it is also rich in calcium and phosphorus, vitamins and enzyme**s (Dillon, 1989).** It is a food that is very suitable for the nutritional and physiological needs of all ages. Due to its nutritional value, the product is suitable for a healthy and balanced diet. The nutritional properties of milk are indisputable, and its protein has significant nutritional value. Their digestion and utilization coefficient and protein efficiency, as well as their biological value, are very high and rank among the best **(Jouan, 2002).**

I.2. Nutritional importance of milk

The nutritional value of milk is extremely high. We need milk from a very young age to build and maintain a strong and healthy body.

Milk contains a lot of nutrients (proteins, carbohydrates, fats, minerals and vitamins), which can be directly absorbed by the body.

The recommendations of the World Health Organization emphasize the importance of

milk and its products for the development and maintenance of health throughout the life stages (childhood, adolescence, and adulthood).

Dairy products are a special food group, and it is recommended to consume 2 or 3 servings a day as part of a balanced diet. [1]

I.3. Composition of Milk

Milk is a complex mixture composed of 90% water, including:

- A real solution containing sugar, soluble protein, minerals and water;
- Soluble vitamins;
- A colloidal solution containing protein, especially casein;
- Emulsion of fat in water **(Courtet Leymarios, 2010).**

The average composition of cow's milk is shown in table 1.

Table 1: Composition of cow's milk.

Main constituent	Range (%)	Mean (%)
Water	85.5 – 89.5	87.0
Total solids	10.5 – 14.5	13.0
Fat (Lipids)	2.5 – 6.0	4.0
Proteins	2.9 – 5.0	3.4
Lactose	3.6 – 5.5	4.8
Minerals	0.6 – 0.9	0.8

I.3.1. Water

Water is the most important ingredient in milk, calculated in proportion. The existence of dipoles and double peaks of free electrons gives it polar characteristics. This feature enables it to form a true solution with polar substances (such as carbohydrates, minerals) and form a colloidal solution with serum hydrophilic proteins. Due to the non-polar (or hydrophobic) nature of fats, they will not dissolve and form an oil-in-water emulsion. The same is true for casein micelles that will form a colloidal suspension, because they are solid. **(Amiot *et al.*, 2002).**

I.3.2. Proteins

The nitrogen content of milk is distributed in casein, whey protein, and non-protein nitrogen (NPN), except for some minor proteins related to FGM. Nitrogen distribution is usually determined by classical Rowland fractionation, which separates casein from whey nitrogen by precipitation at pH 4.6, and separates total protein from whey NPN by precipitation with sodium acetate and acetic acid at pH 5.0. According to this procedure, the average milk nitrogen distribution is approximately 76% casein, 18% whey protein, and 6% NPN. The operational classification of this protein is still used for research and process control. However, the milk protein naming, classification and methods committee of the American Dairy Science Association (ADSA) has developed a milk protein classification system based on amino acid sequence (table 3). The amino acid distributions of the main milk proteins are summarized in table 2 **(Johnson, 1974).**

Table 2: Chemical composition of the major proteins occurring in milk **(Johnson, 1974).**

Acid	α_{s1}-Casein B	α_{s2}-Casein A	k-Casein B	β-Casein A^2	Ɣ-Casein A^2	Ɣ-Casein A^2	Ɣ-Casein A^2	β-Lacto-Globulin A	α-Lact-Albumin B
Asp	7	4	4	4	4	2	2	11	9
Asn	8	14	7	5	3	1	1	5	12
Thr	5	15	14	9	8	4	4	8	7
Ser	8	6	12	11	10	7	7	7	7
SerP	8	11	1	5	1	0	0	0	0
GIu	24	25	12	18	11	4	4	16	8
GIn	15	15	14	21	21	11	11	9	5
Pro	17	10	20	35	34	21	21	8	2
GIy	9	2	2	5	4	2	2	3	6
Ala	9	8	15	5	5	2	2	14	3
ViCys	0	2	2	0	0	0	0	5	8
VaI	11	14	11	19	17	10	10	10	6
Met	5	4	2	6	6	4	4	4	1
Lie	11	11	13	10	7	3	3	10	8
Leu	17	13	8	22	19	14	14	22	13
Tyr	10	12	9	4	4	3	3	4	4
Phe	8	6	4	9	9	5	5	4	4
Trp	2	2	1	1	1	1	1	2	4
Lys	14	24	9	11	10	4	3	15	12
His	5	3	3	5	5	4	3	2	3
Arg	6	6	5	4	2	2	2	3	1
Pyr or GIu	0	0	1	0	0	0	0	0	0

Table 3: Classification and distribution of the milk proteins genus bos (30-35 g/l) **(Johnson, 1974).**

I. Caseins (24-28 g/L)
A. α_{s1}-Caseins (12-15 g/L)
    1. αs1-Casein X^a-8P (genetic variants—A, B, C, D-9P, and E)
    2. αs1-Casein X^a-9P (genetic variants—A, B, C, D-10P, and E)
    3. αs1-Casein fragments[c]
B. αs2-Caseins (3-4 g/L)
    1. αs2-Casein X^a-10P (genetic variants—A, B, C-9P, and D-7P)
    2. αs2-Casein X^a-11P (genetic variants—A, B, C-10P, and D-8P)
    3. αs2-Casein X^a-12P (genetic variants—A, B, C-IlP, and D-9P)
    4. αs2-Casein X^a-13P (genetic variants—A, B, C-12P, and D-10P)
C. β-Caseins(9-ll g/L)
    1. β-Casein X^a-5P (genetic variants—A^1, A^2, A^3, B, C-4P, D-4P, and E)
    2. β-Casein X^a-1P (f 29-209) (genetic variants—A^1, A^2, A^3, and B)
    3. β-Casein X^a-(f 106-209) (genetic variants—A^2, A^3, and B)
    4. β-Casein X^a–(f 108-209) (genetic variants—A and B)
    5. β-Casein X^a-4P (f l-28) [b]
    6. β-Casein X^a-5P (f l-105)[b]
    7. β-Casein X^a-5P (f l-107)[b]
    8. β-Casein X^a-1P (f 29-105)[b]
    9. β-Casein X^a-1P (f 29-107)[b]
D. k-Caseins (2-4 g/L)
    1. K-Casein X^a-1P (genetic variants—A and B)
    2. Minor K-Casein X^a-1, -2, -3, etc. (genetic variants—A and B)
II. Whey proteins (5-7 g/L)
A. β-Lactoglobulins (2-4 g/L)
    1. β-Lactoglobulins X^a (genetic variants—A, B, C, D, Dr, E, F, and G)
B. α-Lactalbumins (0.6-1.7 g/L)
    1. α-Lactalbumin Xa(genetic variants—A and B)
    2. Minor α-Lactalbumins
C. Bovine serum albumin (0.2-0.4 g/L)
D. Immunoglobulins (0.5-1.8 g/L)
    1. IgG immunoglobulins
        - IgG_1 immunoglobulins
        - IgG_2 immunoglobulins
        - IgG fragments
    2. IgM immunoglobulins
    3. IgA immunoglobulins
        - IgA immunoglobulins
        - Secretory IgA immunoglobulins
    4. IgE immunoglobulins
    5. J-chain or component
    6. Free secretory component
III. Milk fat globule membrane (MFGM) proteins
A. Zone A (MFGM) proteins
B. Zone B (MFGM) proteins
C. Zone C (MFGM) proteins
D. Zone D (MFGM) proteins

IV. Minor proteins A. Serum transferrin B. Lactoferrin C. β_2-Microglobulin D. M_1-glycoproteins E. M_2-glycoproteins F. α_1-AcId glycoprotein or orosomucoid G. Ceruloplasmin H. Trypsin inhibitor I. Kininogen J. Folate-binding protein (FBP) K. Vitamin B_{12}-binding protein
[a] X represents the generic variant. [b] Genetic variants of these fragments have not been specifically identified. [c] Nomenclature have not been established for these fragments.

I.3.2.1. The casein

Casein is the group name of the main protein category in milk. Normal milk contains about 3.5% protein, of which casein accounts for about 80%. By acid precipitation or the addition of rennet, casein is easily separated from milk. In cheese making, most casein is recovered together with milk fat. Casein can also be recovered from skimmed milk as a separate product. Casein is dispersed in milk in the form of micelles. The micelles are stabilized by K-casein. Casein is hydrophobic, but K-casein contains a hydrophilic part called glycomacropeptide, which stabilizes the micelles. The structure of micelles is not fully understood. When the pH of milk changes, the acidic or basic groups of the protein will be neutralized. At a pH value where the positive charge of the protein is exactly equal to the negative charge, the net total charge of the protein is zero. This pH is called the isoelectric point of the protein (casein has a pH of 4.6). If acid is added to milk, or if acid producing bacteria are allowed to grow in milk, the pH will drop. As the pH drops, the charge of the casein decreases and precipitates. Therefore, milk coagulates when it becomes sour, or casein precipitates more completely at low pH **(Cortes, C).**

I.3.2.2. The whey proteins

Other milk proteins are found in whey serum. Whey protein is defined as the soluble protein in whey after casein precipitation at pH 4.6 and 20°C (**Dewitt, 1981).** Serum proteins include the first protein fraction (80%) composed of β-lactoglobulin (β-LG), β-lactalbumin (-LA Da), bovine serum albumin (BSA) and immunoglobulin. The second non-protein fraction (20%) is composed of proteases, peptone, and nitrogen-containing compounds **(Fillion, 2006).**

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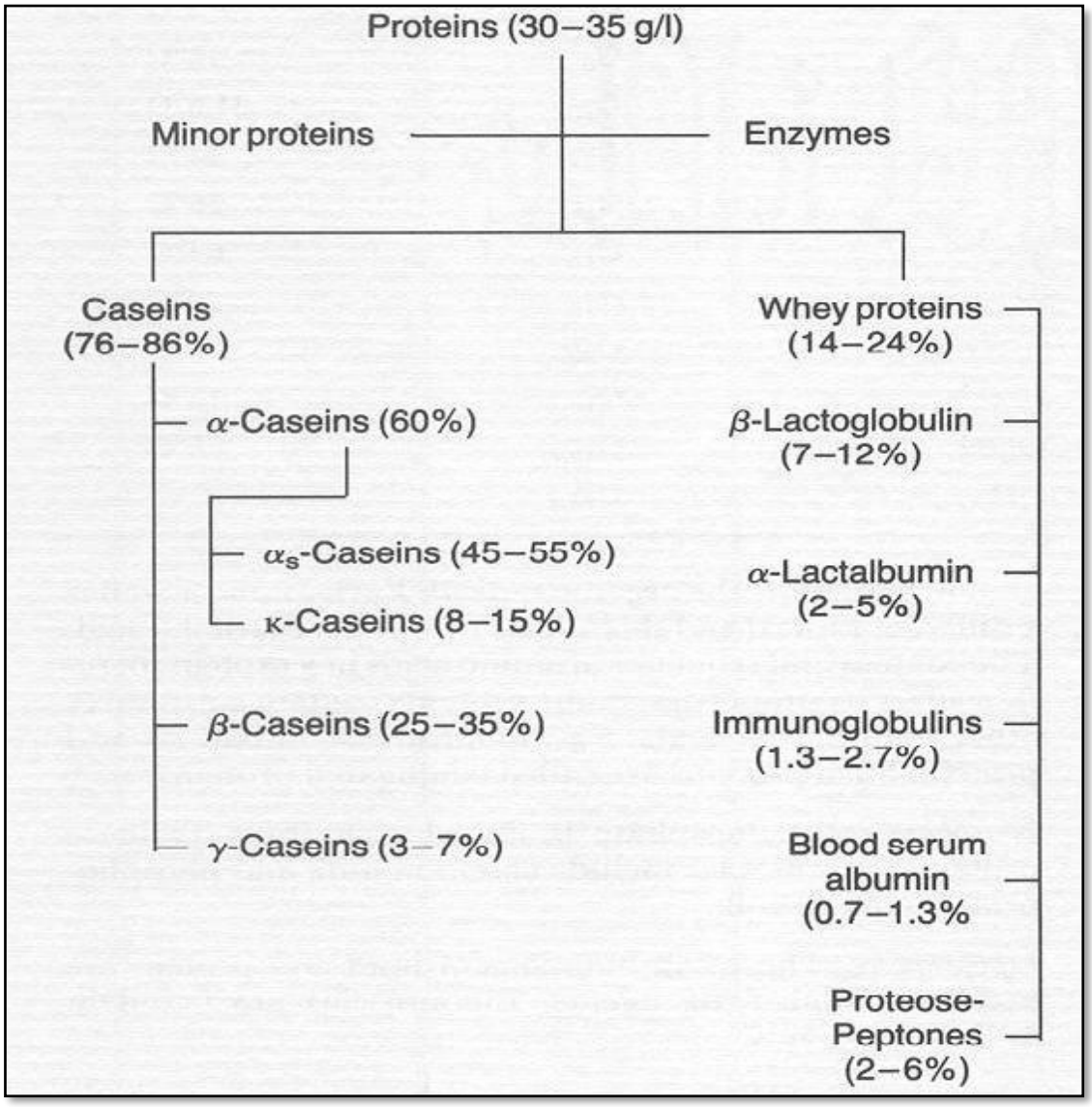


Figure 1: Milk protein fractions.

I.3.2.3. Other milk proteins

In addition to the main protein parts outlined, milk also contains a variety of enzymes. The main enzymes present are lipase, which causes rancidity, especially in homogenized milk, and phosphatase, which catalyzes the hydrolysis of organic phosphates. Measuring the inactivation of alkaline phosphatase is a way to test the pasteurization effect of milk. There are also peroxidases that catalyze the decomposition of hydrogen peroxide into water and oxygen. Lactoperoxidase can be activated and used to preserve milk. Milk also contains protease and whey protein, bovine serum albumin, immunoglobulin and lactoferrin, which catalyze proteolysis, which can protect calves from infection **(Cortes, C).**

I.3.3. Lipids (Fat)

Milk fat is mainly composed of triacylglycerols (about 98 % of total fat), with a small amount derived from phospholipids, cholesterol, free fatty acids, and monoacylglycerol and diacylglycerol. There are also trace amounts of β-carotene, fat-soluble vitamins (A, D, E, and K), and flavoring compounds. Milk fat has a wide range of melting temperatures, from -35 to +38°C, and most of the fat melts between 10 and 20°C. Due to the complex mixture of triacylglycerols in milk fat, these melting characteristics can significantly affect the functional properties of dairy products. The ideal flavor of milk fat is one of its main attributes. The flavor profile is complex, and a large number of volatile compounds contribute to the overall aroma and taste. Two kinds of deterioration reactions occur in milk fat, namely oxidation and lipolysis. These reactions can produce off-flavors, which can destroy the pleasantly delicate flavor of milk fat **(Taylor and Macgibbon, 2002).**

Table 2: Main classes of lipids in milk **(Walstra and Jenness, 1984).**

Lipid class	Amount (wt %)
Triacylglycerols	98.3
Diacylglycerols	0.3
Monoacylglycerols	0.03
Free fatty acids	0.1
Phospholipids	0.8
Sterols	0.3
Carotenoids	trace
Fat-soluble vitamins	trace
Flavour compounds	trace

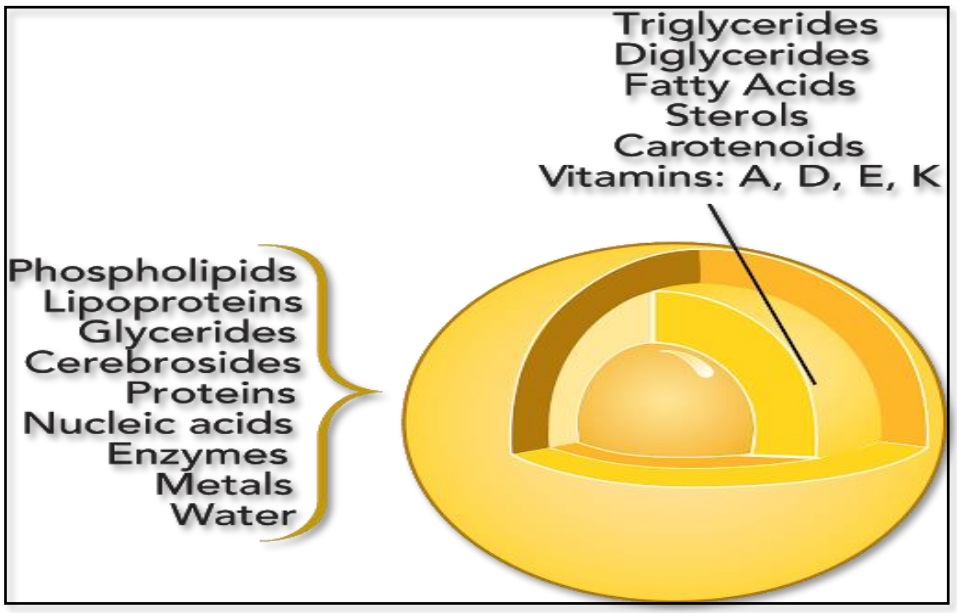


Figure 2: Composition of milk fat **(Bylund, 1995).**

I.3.4. Lactose

Lactose is the main carbohydrate in milk. It is formed by the combination of a molecule of D-galactose (participated by its half-acetyl function) and a molecule of D-glucose (participated by its 4 hydroxyl group). It has a β-galactoside 1.4 bond (hydrolyzed by β-galactosidase) and is 4-D-glucopyranosyl-β-D-galactopyranoside. Although lactose is a sugar, it has no sweet taste. Its concentration is slightly different in milk (4.5 to 5.2 g/100 g). Contrary to fat concentration, the concentration of lactose is not easily changed by the real steps of feeding and dairy competition **(Fillion, 2006).**

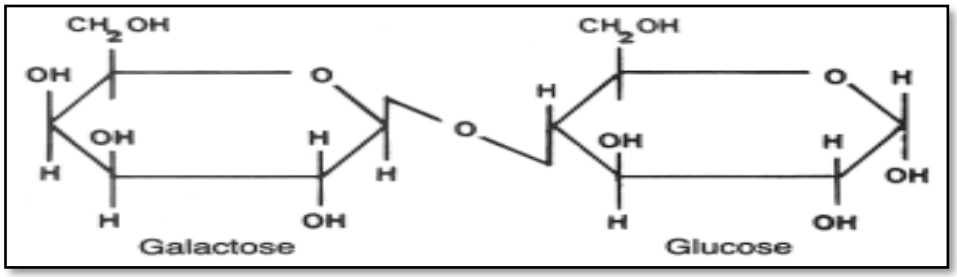


Figure 3: Structure of a lactose molecule.

Lactose is fermented by many microorganisms and is the origin of several types of fermentation that can be used to make dairy products **(Morrissey, 1995).**

a- Lactic acid fermentation: The conversion of lactose into lactic acid due to natural or added lactic acid bacteria (lactic acid fermentation). This lactic acid fermentation is usually

accompanied by the production of more or less large amounts of secondary substances (such as diacetyl), which are responsible for the aroma of dairy products **(Gordon and Loisel, 1991).**

b- Propionic acid fermentation: As propionic acid bacteria convert lactose into propionic acid and acetic acid, which produces the flavor of cooked cheese, carbon dioxide causes the opening of these cheeses **(Luquet, 1985).**

c- Butyric acid fermentation: The lactic acid produced by *Clostridium* is used to convert it into butyric acid, which is responsible for the rot and pungent taste, as well as carbon dioxide and hydrogen. These substances can cause cheese, especially the late expansion of the cooked dough.

d- Alcohol fermentation: As yeast hydrolyzes lactose glucose and galactose, and then converts glucose into ethanol. This fermentation is particularly used to make kefir, a beverage derived from the fermentation of milk, containing a small amount of alcohol and a small amount of carbon.

At high temperatures, lactose and protein participate in non-enzymatic browning, which changes the color of sterilized milk **(Alais, 1975).**

I.3.5. Minor Components

The content of some trace components in milk are listed in table 05, including some vitamins, minerals, non-protein nitrogen compounds, phosphate esters, ethanol and some acids. **(Hui, *et al*., 1992).**

Table 3: Some minor components in fresh milk results are expressed as contents per liter.

Minerals		**Vitamins**	
Sodium (mg)	350-900	**A (jig RE)**	400
Potassium (mg)	1100-1700	**D(IU)**	40
Chloride (mg)	900-1100	**E(JJLg)**	1000
Calcium (mg)	1100-1300	**K(JJLg)**	50
Magnesium (mg)	90-140	**B1 (jig)**	450
Phosphorus (mg)	900-1000	**B2 (JJLg)**	1750
Iron (|xg)	300-600	**Niacin (jig)**	900
Zinc (jig)	2000-6000	**B6 (M^g)**	500
Copper (|xg)	100-600	**Pantothenic acid (jxg)**	3500
Manganese (|xg)	20-50	**Biotin (jig)**	35
Iodine (u,g)	260	**Folic acid (jxg)**	55
Fluoride (|xg)	30-220	**B12 (^g)**	4.5

Selenium (|xg)	5-67	**C(IiIg)**	20
Cobalt ([Lg)	0.5-1.3		
Chromium (|xg)	8-13		
Molybdenum (jig)	18-120		
Nickel (jLg)	0-50		
Silicon ([ig)	750-7000		
Vanadium (jxg)	Tr-310		
Tin (jig)	40-500		
Arsenic (|xg)	20-60		
Selected Miscellaneous Compounds		**NPN Compounds**	
Ethanol (mg)	3	**Total NPN (mg)**	229-308
Formic acid (mg)	10-85	**Urea-N (mg)**	84-134
Acetic acid (mg)	3-50	**Creatine N (mg)**	6-20
Lactic acid (mg)	34-104	**Uric acid-N (mg)**	5-8
Citric acid (mg)	1750	**Orotic acid N (mg)**	12-13
Phosphoric esters (mg)	300	**Peptides N (mg)**	32
Nucleic acids and Nucleotides (mg)	555	**Ammonia N (mg)**	3-14
		Amino acid N (mg)	39-51
		Choline (mg)	43-285
		Carnitine (mg)	10-17
		Af-Acetylneuraminic acid (mg)	120-270

I.3.5.1. Vitamins

Milk contains water-soluble vitamins thiamin (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), vitamin B6 (pyridoxine), vitamin B12 (cobalamin), vitamin C and folic acid. Milk is a good source of thiamine, riboflavin and vitamin B12. Milk contains small amounts of niacin, pantothenic acid, vitamin B6, vitamin C, and folic acid, and is not considered the main source of these vitamins in the diet. It contains fat-soluble vitamins A, D, E, and K related to the fat part, and water-soluble vitamin B complex and C related to the water phase. Vitamins are unstable, so processing will reduce the effective vitamin content of milk. [2]

I.3.5.2. Minerals

Milk is rich in minerals such as calcium, sodium, potassium, iron, magnesium and phosphorus. These minerals are essential to the normal functioning of the body because they are involved in many important processes, such as muscle and heart contraction, blood clotting, hormone release, nerve impulse transmission, etc. Milk contains very small amounts of other minerals called trace elements. They are iodine, fluorine, chlorine, zinc, cobalt and selenium.

In general, mineral elements account for about 4% of body weight. A balanced diet can provide a daily intake of minerals and trace elements. [3]

I.3.5.3. Enzymes

According to **(Fillion, 2006).** Milk contains many enzymes, some of which are oxidases. This is the case with lactate dehydrogenase, malate dehydrogenase and xanthine oxidase Lactoperoxidase and sulfhydryl oxidase. Their role in redox reactions in milk.

As another minor component, milk contains dissolved gases. The oxygen content of raw milk is about 6 mg/L; this presence will have a negative impact on the nutritional quality of the product.

I.4. Factors affecting milk composition

- Breed – generally, high-producing breeds such as Holstein tend to have less milk solids than lower-producing breeds such as Jersey or beef breeds.
- Genetics – within breeds, the selection of bulls with high percentages of solids is essential to maintain the composition of the herd's milk.
- Age – milk fat and protein both decrease with subsequent lactations.
- Nutrition – lactose is a driving factor in milk production, and its percentage has nothing to do with nutritional effects. The milk fat percentage depends on forage intake, fat intake and weight loss. The protein percentage can be controlled by changing the concentrated feed level, starch concentration and amino acid balance.
- Health status – udder health is essential for maintaining optimal milk composition, and the overall health and energy balance of the cow plays an important role in milk quality.[4]

And according to (**Cortes, C):**

- Interval between milkings – the fat content of milk in morning and evening milking varies greatly, because the interval between morning and evening milking is usually much shorter than the interval between evening and morning milking. If a cow is milked every 12 hours, the change in fat content between milkings is negligible, but this is not feasible on most farms. Generally, even if the milking interval is different, the solid non-fat content hardly changes.
- Lactation stage – the fat, lactose and protein content of milk varies according to the stage of lactation. The non-fat solids content is usually highest in the first 2 to 3 weeks,

and then slightly decreases thereafter. The fat content is very high immediately after calving, but quickly begins to decline and lasts for 10 to 12 weeks, and then tends to rise again until the end of lactation.

- Milking completeness – the first udder milk is low-fat, while the last milk (or stripping) is always fairly high-fat. Therefore, before taking a sample for analysis, it is necessary to thoroughly mix all the milk removed. Fat left in the udder at the end of milking is normally collected in the following milkings, so there is no net fat loss.

I.5. Physical properties of milk

Milk is a dilute emulsion consists of a dispersed oil/fat phase and a continuous phase of hydrocolloid. The physical properties of milk are similar to those of water, but they are changed by the presence of various solutes (protein, lactose, and salt) in the continuous phase and the degree of dispersion of emulsification and colloidal components. The physical characteristics of milk are important because these parameters affect the design and operation of dairy processing equipment (such as thermal conductivity or viscosity) or can be used to determine the concentration of specific components in milk (such as using altitude during freezing to estimate addrd water or specific gravity to estimate non-fat solids), or assess the degree of biochemical changes in the milk during processing (for example, starter acidification or curd formation). [5]

Table 4: Common physical constants of cow's milk **(Luquet, 1985).**

CONSTANTS	VALUES
pH (20°C)	6,5 to 6,7
Titratable acidity (°D)	15 to 18
Density	1,028 to 1,036
Freezing point (°C)	(-0,51) to (-0,55)
Boiling point (°C)	100,5

I.5.1. Density and specific gravity

Milk is a complex colloidal system. The colloidal system includes a dispersion medium, water, and a solution containing salt and sugar. Therefore, it is heavier than water **(Eckles *et al.*, 1951).**

The density of milk is the weight per unit volume at a specified temperature. Specific gravity is the density of a substance divided by the density of water at the same temperature. It is basically 1.000 at 4°C. The water, defatted solids and fat content, and the degree of hydration of fat crystals or protein are determined by using specific gravity. When the temperature rises, the density of milk will decrease. Temperature control is very important during the measurement. The specific gravity of water is lower than that of milk. When water is added to milk, the specific gravity will decrease. However, a higher content of milk fat showed the same effect. Specific gravity is one of the most practical measurements used to control the composition of milk. The specific gravity of whole milk is 1.032, skimmed milk is 1.036, and diluted whole milk is 1.066 **(Rosenthal, 1991).**

Milk specific gravity is typically measured in the laboratory at 60°F (15.5°C). The composition of the milk affects the specific weight of the milk. The components of the milk vary in specific gravity, approximately: fat - 0.93; lactose, 1.666; proteins, 1,346; casein, 1.31; salts, 4.12 **(Eckles *et al.*, 1951).**

The density is so variable due to all the substances included in the composition. The density of the milk is 1.027 g/ml - 1.035 g/ml at 20°C due to its composition. The density of milk decreases due to the increase in fat content and increases due to the decrease in fat content. Milk density increases due to increasing amounts of protein, lactose and minerals. The increase in temperature causes the density of milk to decrease **(Metin, 2001).**

The specific gravity of the milk is 1.021–1.037. Milk density typically ranges from 1.028 to 1.034 depending on the composition. Thus, milk is very slightly thicker than water **(Neville and Jensen, 1995).**

I.5.2. pH or actual acidity

The pH of milk determines whether it is acid or alkali. Milk is slightly acidic or close to neutral pH. The exact value of the sample depends on when the cow produced milk, any processing performed on the milk, and when it was packaged or opened. Other compounds in milk act as buffers, so mixing milk with other chemicals will bring their pH closer to neutral.

The pH of a glass of cow's milk is between 6.4 and 6.8. Fresh milk from the cow typically has a pH between 6.5 and 6.7. The pH of the milk changes over time. When the milk turns sour, it becomes more acidic and the pH drops. This happens when the bacteria in the milk convert the sugar lactose into lactic acid.

The first milk a cow produces contains colostrum, which lowers its pH. If a cow has mastitis, the milk pH will be higher or more basic **(Helmenstine, 2019).**

I.5.3. Titratable acidity or dornic acidity

In practice titratable acidity is usually used in milk and dairy products. It is defined as the buffer capacity between its own pH and pH ≈ 8.3; it is usually expressed in °N = mmol NaOH per liter of milk or milk product. The average contributions of milk components to the acidity are approximately:

2.2 °N per % casein, thus on average	~5.7 °N
1.4 °N per % serum protein	~0.9 °N
0.1 °N per mM colloidal inorganic phosphate	~1.0 °N
0.7 °N per mM dissolved inorganic phosphate	~7.8 °N
1.5–2 °N for other compounds	~1.7 °N
Total average	~17 °N

In most fresh milk samples the titratable acidity is in the range of 14 to 21°N (average around 17°N), usually at the beginning of lactation it is high, say 3°N above the level reached later. The pH of most milk samples is 6.6 to 6.8; an average of 6.7 at 20°C. This means that the activity of H+ ions ranges from 0.16 to 0.25 µmol·l -1. Titratable acidity and pH show a weak negative correlation **(P. Walstra, Pieter Walstra, Jan T. M. Wouters, Tom J. Geurts, 2005).**

I.5.4. Freezing Point or cryoscopic constant

Neville and Jensen (1995) proved that the freezing point of milk is slightly lower than that of pure water because the presence of dissolved solids lowers the freezing point. This physical property is measured to determine if there is additional milk water. Its average value ranges from -0.54 to -0.55°C, which is also the blood serum freezing point. There are slight variations with the seasons, breed of cows and the area of production. For example, there have been reports of normal fluctuations from -0.530 to -0.575 degrees Celsius. Mooring brings the freezing point to 0°C as the number of non-water molecules and ions per liter is reduced. In general, all treatments or changes in milk composition that change the amount of milk result in a change in the freezing point **(Mathieu, 1998).**

I.5.5. Boiling point

The boiling point is defined as the temperature reached when the vapor pressure of a substance or solution is equal to the pressure applied. Thus, as with the freezing point, the boiling point is affected by the presence of dissolved solids. It is slightly higher than the boiling point of water, i.e. 100.5°C **(Amiot *et al.*, 2002).**

I.6. Undesirable components of milk

Milk may contain substances that the animal swallows or breathes in, both as primary ingredient and as metabolized compounds. Foreign substances can come from the diet (fertilizers and phytosanitary products), from the environment assigned to the animal (pharmaceuticals, antibiotics, hormones) **(Mahieu *et al.*, 1977).**

I.6.1. Antibiotics

Antibiotic residues, especially when these substances are applied topically to treat mastitis **(Jacquet, 1969),** their presence in milk causes a double drawback. Thus, for the consumer, it may be responsible for allergic and carcinogenic phenomena **(Michell, 2005).** In sensitive people, it may contribute to the installation of endogenous antibiotic flora **(Morel, 1962).**

I.6.2. Pesticides

Pesticide residues are polychlorinated fat-soluble substances and therefore accumulate in fat reserves. When fat melts, the stored substances are suddenly released back into circulation and intoxication can occur **(Beroza and Bowman, 1996).**

I.6.3. Metals

Metals that can contaminate milk at health-threatening levels include selenium, arsenic, lead and mercury **(Vanier, 2005).**

The quality of milk

II. The quality of milk

II.1. Organoleptic quality

The evaluation of sensory quality is a subjective operation because it focuses on the following characteristics: smell, color, taste and flavor, which are all characteristics of milk **(Boudelia *et al.*, 2016).**

II.1.1. The colour

Due to light scattering from fat globules and casein micelles, milk appears cloudy and opaque. **[6]**

The white color of milk is one of its most unique characteristics. Milk reflects all wavelengths of light and does not absorb any color due to its reflective properties. The particles present in milk such as casein, calcium complex and fat are all white **(Aaisha, 2017)**

II.1.2. The flavour

Good flavor is an indispensable quality mark of beverage milk. Fresh milk has a fairly mild flavor, and whole milk has a stronger flavor than (partially) skimmed milk. However, the main aspect is that there is no peculiar smell. Fresh milk may already have a peculiar smell **(Walstra *et al.*, 2006).**

The natural sweetness of milk is due to the combined effect of its ingredients. Due to a variety of factors, the peculiar smell in milk will quickly develop. The feed eaten by animals may cause some unpleasant flavors. Bacterial growth in milk can produce fruity, barn, malt, or sour flavors. Enzymatic activity may also cause unnatural taste. Rancidity due to the action of lipase is a typical example. The oxidation reaction may cause a cardboard smell in the milk. Milk processing may produce a cooked taste.

Milk usually has a slightly sweet flavor. Occasionally, some unhealthy salt, bitter, sour, rancid, feed and other peculiar smells, peculiar smells, milk smells, etc. are caused by various factors **(Roberts, 1993).**

- **Classification of off-flavours**

The common odors in milk can be divided into three basic categories **ABC** of odor development.

Absorption - feed, barn, cattle, dirty, weed and musty smell.

Bacterial - acid, malt, unclean, fruity and rotten.

Chemical - cows (ketosis), rancid, oxidized, solar and healing.

- Rancid flavor

Soapy bitterness can be distinguished from rancidity. There seems to be a seasonal effect, with the highest incidence in the months between July and September. Rancidity is caused by chemical development, which continues until the milk is pasteurized.

- Farm oxidized flavor

The oxidized taste is also a reaction of the milk fat. Carton or metallic flavored milk is more common in milk in winter and early spring. An unpleasant aftertaste can be detected in raw milk, but sometimes up to two days after intake.

- Feed flavor

All the flavors of the feed are absorbed directly into the milk by the cow's system. Cows emit smell and taste within 30 minutes of eating or breathing the silage. It gets stronger roughly after an hour.

The smell and taste of grass or corn silage, legume hay, and beer grains are the ingredients that cause most problems. Two ways the smell can be transferred to milk are:

Nose or mouth → lungs → blood → milk

Mouth → digestive tract → blood → milk

- Unclean flavor

Dust, dirt, and feces can cause an unclean taste of milk. Cows and their surrounding environment must be kept clean. Milking equipment that is not properly cleaned and disinfected may be a factor.

Some farm milk samples have an unpleasant, dirty aftertaste. This problem often occurs in winter. Usually it is an absorbed taste, such as silage. Usually cows breathe the air with the smell of barn and transfer it to the milk.

- Malty

Malty flavor is usually the precursor of high sourness. It rarely appears in pasteurized milk. However, the characteristic flavor after processing will still be retained, despite the flavor produced in the raw milk. If it is not stopped by pasteurization, the malt flavor will become highly acidic later.

Malty tastes like grape nut cereal. The reason is that lactic acid *Streptococcus* is present in poorly cooled milk. The problem can be exacerbated when milk in one bulk tank is mixed with milk in many other tanks in truck tanks and then mixed in factory raw milk storage tanks. Milk samples from bulk tanks on the farm have not been cooled for 12 hours, and their bacterial counts are always high.

- High acid

For fluid or manufacturing purposes, no bacterial flavor is allowed in milk. Hygiene is the key to preventing high sourness. Spoilage is due to the effect of bacteria on lactose (milk sugar).

- Putrid

The taste produced by psychrotrophic bacteria is usually described as old, lack of freshness, fruity, bitter, fermented or rotten taste. Usually titratable acidity may be close to normal.

The rotten taste is the result of bacterial contamination, storage temperatures above 40°F, and aging. The deterioration of milk is due to the effect of bacteria on protein rather than lactose. If left for a few days, the rotten milk will coagulate, separate and may give off a rotten smell.

Raw milk collected from the farm may have a rancid smell due to contamination and storage for three or four days. Sometimes, retail milk samples can be found to have a rancid smell and an excellent number of bacteria **(Robert and Virginia, 2016).**

II.1.3. The taste

Consumers judge milk quality mainly by taste and appearance **(Roberts, 1993).**

Milk flavor, as the word is commonly used, is the sensation felt when milk is ingested into the mouth. Basal milk taste was defined as the sum of all taste sensations from normal milk that is unaffected by the feed or the secretion of abnormal milk.

As already stated, those components of the milk which are naturally tangible must have a significant influence on the original taste of the milk. It is believed that the presence as well as the amount of these substances is important **(Bloksma *et al.*, 2008).**

II.1.4. The smell

Fresh milk will never smell bad. The smell is characteristic of milk because the fat contained in it preserves animal smells. They are related to the milking, food and storage atmosphere **(Vierling, 2003).**

II.2. Microbiological quality

Milk is practically sterile when it is synthesized in healthy cows' udders (mammary gland). Cows, like humans, are natural reservoirs of bacteria.

Milk is a good source of nutrients and edible energy, not only for mammals, but also for the numerous microorganisms that can thus thrive in the milk. This primarily affects bacteria, but some molds and yeasts can also grow in it **(Walstra *et al.*, 2006).**

The main microorganisms present in milk are bacteria. But we can also find yeasts and molds and even viruses. Many species of bacteria can grow in milk, which is an excellent substrate for them **(Institut de l'élevage, 2009).**

Microorganisms can enter milk through cows, air, feed, milk processing equipment and milking machines. Once the microorganisms enter the milk, their number will increase rapidly **(Cortes, C).**

Microorganisms in milk are divided into two categories according to their importance: endogenous or primitive flora and contaminated flora **(Vignola, 2002).**

II.2.1. Original flora

Milk obtained from good conditions from a healthy animal contains few microorganisms (less than 103 germs/ml). When it comes out of the udder, it is practically sterile and protected by inhibitory substances called lactenin's for a time-limited activity (about an hour after milking) **(Cuq, 2007).**

The original flora of dairy products is defined as all the microorganisms found in the milk at the udder outlet, and the dominant genera is mainly mesophilic bacteria. These are *Micrococcus,* but also *Streptococcus lactis* and *Lactobacillus* **(Vignola, 2002).**

These microorganisms (*Micrococci*, but also Lactic *Streptococci* and *Lactobacillus*) are more or less numerous and closely related to food **(Guiraud, 2003).**

Milk secreted from the udder of a healthy animal contains relatively little microorganisms. It should contain less than 5,000 CFU. The natural flora of raw milk is an important factor, especially in these organoleptic properties **(Fotou *et al.*, 2011).**

Table 5: Original flora of milk **(Vignola, 2002).**

Microorganisms	**Percentage %**
Micrococcus sp	30-90
Lactobacillus	10-30
Streptococcus or Lactococcus	<10
Gram negative	<10

II.2.2. Contamination flora

Milk is sterile in the udders of healthy animals, but is mainly contaminated with bacteria during and/or after milking **(Karimuribo *et al.*, 2005; Mugampoza *et al.*, 2011).**

Microbial contamination in milk comes from the milk itself, because it can be naturally contaminated, or it can come from infected or diseased animals, humans, the environment, water and equipment used for milking and storing milk. These sources of contamination include disease-causing organisms (pathogens) shed from milk, infected udder and/or teats, animal skin, fecal udder contamination, contaminated milking and storage equipment, and water used for cleaning. Other sources of bacteria come from the air, milking workers, handlers, drugs or chemicals used in handling animals, and water used for adulteration by unethical and unfaithful workers/sellers, which may be contaminated and may cause other health problems **(Karimuribo *et al.*, 2005; Swai and Schoonman, 2011).**

Generally speaking, when milk is contaminated by a variety of factors, the quality of milk may be reduced, such as adulteration, contamination during and after milking, and the presence of breast infections, mastitis (inflammation of the mammary gland) disease, and use for the treatment of diseases of drug residues. Considered public health **(Karimuribo *et al.*, 2005; Syit, 2008; Mdegela *et al.*, 2009).**

II.2.2.1. Pathogenic flora

The most important bacteria in this pathogenic flora are usually mesophilic bacteria. The main pathogenic microorganisms related to dairy products are: *Salmonella, Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Bacillus cereus,* and small intestine *Yersinia* inflammatory disease, *Listeria monocytogenes, Escherichia coli, Campylobacter jejuni, Shigella Sonnei* and some molds **(Vignola, 2002).**

II.2.2.2. Alteration flora

This flora takes advantage of sensory defects (taste, aroma) or defects that will shorten the shelf life of dairy products. There are three types of spoilage flora: coliforms, yeasts and molds **(Essalhi, 2002).**

Coliform bacteria are mainly of the genera: *Escherichia* and *Enterobacter*, spore plants such as *Bacillus* sp, *Clostridium* sp **(Vignola, 2002 and Richard, 1990).**

Table 6: Some properties of microorganisms in raw milk.

Microorganisms	Characteristics	Effects	References
Clostridium	Gram positive Strict anaerobes	Contamination of milk at the time of milking	**(Bourgeois 1996).**
Escherichia coli	Mobile pathogenic	Able to ferment glucose and lactose	**(Carip, 2008).**
Salmonella	Pathogenic gram negative mobile pH-sensitive acidic aero-anaerobes facultative	Able to ferment glucose unable to ferment lactose	**(Carip, 2008).**

Staphylococcus	Gram positive motionless not encapsulated non-spore	Able to ferment glucose	**(Carip, 2008).**

II.3. Sources of contamination in milk

The microbial contamination of milk can come from internal and / or external sources.

II.3.1. Interior of udder

Aseptic milk has different numbers of bacteria with a reported number of <100-10,000 CFU/mL from normal udder, but the predicted average is 500-1,000 CFU/mL in developed countries. Microorganisms enter the udder through a canal at the tip of the teat, which varies in length (from 5 to 14 mm) and its surface is heavily keratinized. This keratin layer retains milk residues and has an antibacterial effect.

In the milking process, bacteria are most abundant at the beginning and then gradually decline. This is mainly due to the mechanical movement of bacteria, especially in the teat canal, where this number is likely to be highest. For this reason, the rejection of the first few milk streams helps to reduce the number of microorganisms in the milk. Milk from different quarters also differs in number. The different species of bacteria that are found in milk because it comes from the udder are very limited

Although the *Micrococci* grow slowly, if allowed to grow, they do cause acid formation and proteolysis. They are mostly non-pathogenic. *Streptococci* are less common than micro clusters. *Streptococcus agalactiae* can even be present in non-clinical mastitis and therefore appears to be a natural inhabitant of the udder. Among the gram-positive rods, *Corynebacterium bovis* was found in large numbers. It is non-pathogenic, but if bred it causes rancidity. If an animal is infected with mastitis, microbial contamination from the animal's udder contributes in particular to the total number of microorganisms in the bulk milk compared to that of a healthy animal. The effect of mastitis on the total number of bacteria in milk depends on the type of infectious organism. The most common pathogens causing mastitis in milking animals (Figure 4) are *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Escherichia coli,* and *Corynebacterium pyogenes.*

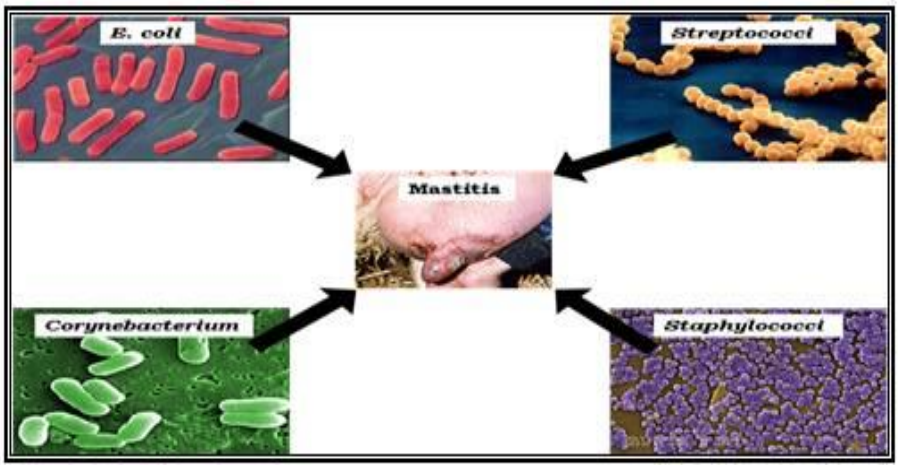


Figure 4: Most common microbial agents of mastitis.

II.3.2. Exterior of udder

In addition to udder infections, unclean animal udders and teats also contribute significantly to the total amount of bacteria in milk. The majority of microorganisms in milk are naturally associated with the skin of animals, as well as those originating from the environment in which the cow is kept and milked. Environmental conditions such as soil, manure, mud, feed, or mulch; determines which microorganisms will dominate the milk. The udders and teats become soiled with feces, mud, litter such as sawdust, straw, etc. For heavily soiled teats, this figure can be 100,000 CFU/ml. In winter, the bedding material contains a large number of bacteria, mainly psychrotrophs, coliform bacteria and *Bacillus* spp simple washing does not have a significant effect on the udder microflora. Sparing washing with sodium hypochlorite combined with drying helps to reduce the number of microorganisms.

The different categories of outdoor microbes she lists are:

- There are mainly *Micrococci* and coagulase negative *Staphylococci*.
- Fecal *Streptococci* then appear on the teat surface, but fewer gram negative bacteria, including coliform bacteria. Coliform bacteria do not survive well on the test surface.
- Aerobic thermoduric organisms are exclusively *Bacillus* spp. B. *licheniformis,* B. *subtilis,* B. *pumilus* are more common, less often B. *cereus,* B. *circulans* and B. *firmus.*

The teat surface can also contain clostridial spores, which are usually found in cow feed, litter and feces. Psychrotrophic and thermoduric bacteria predominate on the teat surface. The psychrotrophs that can grow at 7°C and below are mainly gram-negative rods, the major ones being *Pseudomonas fluorescens,* followed by *Alcaligenes, Flavobacterium,* and coliforms.

On the other hand, thermodurics on teat surfaces are often bacterial spores (dormant and non-reproducing structure; high resistance to radiation, desiccation, lysozymes, heat, hunger and disinfectants) that are usually found in soil. When these spores enter milk in bulk, they can survive during pasteurization and cause a number of post-pasteurization problems.

II.3.3. Coat of cow

The coat serves as a carrier of bacteria directly into the milk. The hair around the udder, sides and tail contribute to more bacteria in the milk. The coat can indirectly transport microbes into the air, especially *Bacillus* spp. Dander can transmit bacteria from standing water pools, especially from weeds that cause milk microbes.

II.3.4. Animal shed and surroundings

Milk produced on farms with poor hygiene practices can deteriorate significantly and have a shorter shelf life than milk produced under hygienic conditions. Microbes associated with bedding material include:

- Coliform bacteria
- Spores
- *Staphylococci*
- *Streptococcus*
- Other gram negative bacteria

II.3.5. Milking staff

Staff involved in the different stages of milk production play a key role in maintaining hygiene and preventing contamination of the milk. Hand contact or the milker's removal of dust and dirt particles can add different types of germs to the milk.

The risk of contamination from the milkman is much higher when cows are milked by hand compared to milking machine. Contaminated clothes and hands increase the risk of contamination of the milk and milking equipment many times over. The milker with infected wounds on the hands contributes to the development of pathogenic *Streptococcus* spp and *Micrococci.* When wet hand milking is practiced, microorganisms present in lubricants such as pre-milk, milk or milker's saliva, and bacteria from hands and teats will end up in the milk. Common human microbial pathogens that cause diseases such as typhoid, paratyphoid and dysentery can contaminate milk.

Microbial pathogens causing scarlet fever, septic sore throat, diphtheria, cholera, etc. contaminate the milk.

II.3.6. Milking equipment

Incorrectly cleaned milking and cooling equipment is one of the main sources of milk contamination. The milk residue left on the contact surface of the equipment supports the growth of various microorganisms. Although natural inhabitants of the teat canal, apex and skin; microbes associated with infectious mastitis do not thrive on these devices, it is possible that some strains associated with environmental mastitis may grow to significant levels. Since it is very difficult to remove any milk residues and sediment from the surfaces that come into contact with the milk of the milking equipment; therefore, equipment with smooth surfaces and minimal welds should be used. The tanker and collection pipes are also potential sources of contamination if not properly cleaned. In addition, biofilms can easily deposit on closed, hard-to-clean surfaces.

Soiled or improperly cleaned milk cans and lids, if still wet, cause the proliferation of thermophilic bacteria such as *Bacillus cereus.* Improperly sterilized milking machines contain thermoduric *Micrococci, Bacillus* spp. and *Microbacterium* spp. mainly compared to coliforms and S*treptococci*. Rubber hoses mainly contribute to *Pseudomonads* rather than thermoduric.

II.3.7. Water supplies

On dairy farms, water can be the main source of microbial contamination. The water used for production should be of good bacteriological quality. Inadequately or untreated storage tanks, untreated water from natural sources such as drilling wells, reservoirs and rivers can also be contaminated with fecal microbes (e.g. coliforms, *Streptococci* and *Clostridia*). In addition, a wide variety of saprophytic bacteria (i.e. *Pseudomonas*, coliforms, other gram-negative rods, *Bacillus* spores, *Coryneform* and lactic acid bacteria) can be present in the water and have the potential to contaminate milk. The warm water used to wash the udder is a powerful source of *Pseudomonas* and cola, which can even cause mastitis.

II.3.8. Airborne contamination

Contamination of milk with airborne bacteria is negligible compared to microorganisms from the teat surfaces. The number of airborne microbes in sheds rarely exceeds 200 CFU/l. *Micrococci* constitute> 50% of the air microflora. Air contains dust, moisture and bacteria; hence its input should be minimized in milk.

Micrococci, Coryneforms, Bacillus spores, *Streptococci* and gram-negative rods are the main types present in the air. In general, more air in milk leads to faster growth of bacteria.

Here are some of the practices that increase the amount of air in milk:

- Sweeping the floors just before the milking process.
- Handling hay and forage shortly before the milking process.
- Brushing the animals before milking.
- Prepare dusty litter for animals.
- Let dust and dirt accumulate on the walls or ceiling of the shed. [6]

II.4. To reduce the risk of contamination

- **Animal cleanliness**
  - All animals should be kept clean.
  - All lying areas should be of an adequate size and should be kept clean and dry.
  - Walkways and access roads should be free from accumulations of manure and slurry.
  - Fields, tracks and gates should be well maintained and free from accumulations of manure, slurry and mud.
- **Milking practices**
  - Each animal's milk must be examined for physical / chemical / organoleptic abnormalities and if any abnormal milk is found, the milk must be discarded.
  - Clean the teats, udders and adjacent parts before milking.
  - Hands, contact surfaces and milking equipment must always be kept clean.
- **Milking equipment**
  - Surfaces in contact with milk must be properly cleaned and disinfected immediately after each milking.
  - All equipment must be kept clean and in good condition.
- **Milk storage and cooling**
  - Milk must be protected from contamination during handling and storage.
  - Milk must be chilled quickly to minimize the multiplication of bacteria.
  - Collection tanks should be cleaned and disinfected after each milk collection and kept in good condition. [7]

II.5. Main activities of microorganisms in milk

Changes in milk are associated with the multiplication of yeast, mold and bacteria. Bacterial infections are the most common and most important, and their potential development is of greatest concern. These degradation processes are possible when the conditions of the surrounding environment favor microbial proliferation and enzymatic activity. Serious taste and odor defects may appear **(Kim *et al.*, 1982).**

II.5.1. Acidification

This process leads to the coagulation of casein and most of the milk. Depending on the temperature of the milk and the bacteria involved, the clotting phenomenon will be more or less violent: from 10°C to 37°C the most commonly involved germ is *Streptococcus lactis,* less commonly associated with coliforms, *Enterococci*, *Micrococci* and *Lactobacilli.*

Above 37°C the germs involved *are Streptococcus thermophilus, Enterococcus faecalis* and *Lactobacillus bulgaricus.*

The process is slower at temperatures below 10°C and the swelling requires a relatively long delay. The clot can be degraded in a second step by proteolytic psychrotrophs species: proteolytic psychrotrophs: *Pseudomonas, Acinetobacter, Micrococci*... **(Guiraud and Galzy, 1980; Leyral and Vierling, 2007).**

II.5.2. Proteolysis

During metabolic activity, some microorganisms break down milk protein fractions due to the action of their proteases. This phenomenon causes the release of a wide variety of products including long and short chain peptides which cause a bitter taste. Infected germs are *Micrococcus, Bacillus, Clostridium, Pseudomonas* **(Vignola, 2002; Guiraud, 2003).**

II.5.3. Lipolysis

Lipolysis is an enzymatic reaction to break down fat that produces milk by increasing the free fatty acid content. Above certain thresholds, this increase can cause off-flavor (rancid) dairy products **(Heuchel *et al.*, 2003).**

In chilled raw milk, the dominant flora is represented by psychrotrophs. 70% or more of this population has lipolytic activity. However, it is not noticeable in the taste only from the content of 10^6 to 10^7 microorganisms/ml, i.e. for raw milk considered to be heavily contaminated **(Chilliard and Lamberet, 1984).**

Experimental part

Material and methods

I. The objective of the study

The main aim of our study is to evaluate and compare the physicochemical and microbiological quality analyses of raw milk collected from two dairies of two different regions: 1st sample from a traditional farm in a rural village called RAS EL AGBA in and the 2nd sample from unauthorized farm that feed cows from garbage containers in Bourwayah district in the city center of Guelma province.

II. Material and methods

II.1 Presentation of the study area

The study carried out in the university laboratory of the Faculty of Natural and Life Sciences and Earth and Universe Sciences of the University 8 May 1945 Guelma.

II.1.1. The geographical location of Guelma

The wilaya of Guelma is located in the north-east of the country and is, from the geographical point of view, a meeting point, even a crossroads between the industrial poles of the North (Annaba – Skikda) and the trading centers in the South (Oum-El Bouaghi and Tébessa), in addition to the proximity of the Tunisian territory to the East.

The territory of the Wilaya is characterized by a sub-humid climate in the center and north and semi-arid towards the south. This climate is mild and rainy in winter and warm in summer. The temperature ranges from 4°C in winter to over 35°C in summer and averages 17.3°C.

Figure 5: Geographical limits of Guelma.

II.1.2. The geographical location of Ras El Agba

Figure 6: Geographical limits of Ras El Agba.

II.2. Material:

- ✓ Autoclave
- ✓ water Bath
- ✓ precision balance
- ✓ Bunsen burner
- ✓ bacteriological oven
- ✓ Incubator
- ✓ Refrigerator (beko)
- ✓ Micropipette 100 µL and 1000 µL
- ✓ platinum loop
- ✓ Optical microscope, Cooler, Hot plate with stirrer; magnetic stirrer
- ✓ Culture media used : (PCA, SS agar, BCPL broth, Chapman agar, M17 agar)
- ✓ Chemical products : Buffered Peptone water, Physiological water, distilled water, hydrogen peroxide, NaOH 1/9 N, phenolphthalein (1%), gentian violet, lugol, diluted fuchsine to 1/10, alcohol 90%, Selenite-cysteine broth, indole-free peptone water, Oxidase discs
- ✓ petri dishes
- ✓ The used glassware: (bottles, graduated pipette, Pasteur pipette, test tubes, Erlenmeyer, beakers, glass slides and cover glasses, Durham Bell)
- ✓ pH meter
- ✓ Milk analyzer

II.3. Sampling

II.3.1. Samples

The physico-chemical and microbiological analyses of milk from two different regions BOURWAYAH and RAS EL AGBA, were carried out on a number of two samples (one sample) for each region.

II.3.2. Sampling techniques

The samples for both microbiological and physicochemical analysis were milked directly from the udder into 250ml reusable sterile glass culture vials with cap. The samples are immediately cooled in a cooler, until the moment of the analysis with a delay not exceeding 8 hours **(Guiraud, 2003).**

II.4. Methods

II.4.1. Physicochemical analysis

II.4.1.1. Boiling test

All milk must be boil-stable.

The test is quick and simple. It is one of the old tests for too acid milk (pH<5.8) or abnormal milk (e.g. colostral or mastitis milk). It is used during milk collection to detect the instability of milk proteins through a simple heat treatment to precipitate them, if the milk sample fails in the test, the milk must contain many acid or rennet producing microorganisms or the milk has an abnormal high percentage of proteins like colostral milk. Such milk cannot stand the heat treatment in milk processing and must therefore be rejected.

Method of operation

- In a test-tube, boil 2 to 5 ml of milk.

Figure 7: Boiling test.

- Expression of results

- If no coagulation occurs, and the milk flows down the sides of the tube without leaving any traces, the milk is normal and it can stand heating operations at the time of testing.
- If clotting, coagulation or precipitation occurs, the milk is abnormal and it has failed the test, acidified milks (at 25°D) coagulate by boiling **(Pougheon, 2001).**

II.4.2. pH measurement

The pH value is defined as the negative common logarithm of the active hydrogen ion H+ concentration in an aqueous solution. Milk is considered fresh if its pH is between [6.4 and 6.8].

Method of operation

- Calibrate the pH using two buffer solutions of pH=4 and pH=7.
- Immerse the electrode in the water to be analysed and read the pH value.
- After each pH determination, remove the electrode, rinse with distilled water and dry.

❖ **Method using the pH meter**

- Expression of results

- The result is displayed directly on the pH meter.

II.4.3. Determination of dornic acidity

The acidity of the milk is titrated with DORNIC soda (NaOH 1/9N) in the presence of a colored indicator phenolphthalein (1%). The end of the reaction is indicated by a coloration (pale pink), persistent for a few seconds.

$$CH_3\text{-}CHOH\text{-}COOH + NaOH \rightarrow CH_3\text{-}CHOH\text{-}COONa + H_2O$$

This acidity is expressed in degree dornic (°D) where: 1°D represents 0.1g of lactic acid in one liter of milk **(Mathieu, 1998).**

Method of operation

- Place 10 ml of the sample in a 100 ml beaker.
- Add 0.3 ml of the 1% phenolphthalein solution to the solution.
- Titrate with sodium hydroxide (NaOH N/9) until the solution turns pink, which should last for about 10 seconds.

❖ **Method using the MilkoScan FT 120**

- Expression of results

- The result is displayed directly on the MilkoScan FT 120

II.4.4. Determination of density

The density of milk is an intrinsic result of its constituents, it depends on their degree of hydration, particularly with regard to proteins **(Hardy, 1987).** The density of milk is the ratio of the masses of the same volume of milk and water at 20°C **(Mathieu, 1998).**

Density has been determined by two different methods:

➢ **The Pycnometer method**

Method of operation

- Weigh the empty pycnometer.
- Fill it with milk at 20°C and avoid any incorporation of air bubbles.
- The pycnometer is weighed a second time.

➢ **Method of Lactodensimeter**

Method of operation

- Homogenize the milk sample.
- Pour the milk into a 250ml test tube, hold it at an angle to avoid the formation of foam or air bubbles (Air bubbles or foam would interfere with the test reading the result).
- Hold the thermo-lacto-densimeter by the tip, lower it gently into the milk with a rotating movement and wait for its stability. Do not let go until it is almost in equilibrium.
- Allow the lactometer to float freely until it reaches equilibrium for thirty seconds to one minute. Then read the lactometer at the top of the meniscus. Immediately, read the temperature of the milk.

❖ **Method using the MilkoScan FT 120**

- Expression of results

- The result is displayed directly on the MilkoScan FT 120

II.4.5. Measurement of total dry matter content

The TDM of milk is the product resulting from the desiccation of milk under the conditions described in the standard **(AFNOR, 1985).**

Method of operation

- Into a dried and tared capsule, introduced with the pipette 3g of milk.
- Place it in the oven set at 103°C ± 2°C and leave it for 3 hours.
- Then put the capsule in the desiccator and let it cool down to room temperature.
- The residue is then weighed with an analytical balance.

❖ **Method using the MilkoScan FT 120**

- Expression of results

- The result is displayed directly on the MilkoScan FT 120

II.4.6. Determination of the defatted dry matter

The DDM is obtained by the difference between the total dry matter and the fat. Normal milk usually contains 90 to 95 (g/l) of non-fat dry matter.

DDM (%) = TDE (%) - FM (%)

DDM: Defatted dry matter.

TDE: total dry extract.

FM: fatty matter.

❖ **Method using the MilkoScan FT 120**

- Expression of results

- The result is displayed directly on the MilkoScan FT 120

II.5. Microbiological analysis

Analysis for microbial quality of raw milk involved count of aerobic mesophilic bacteria, total coliform, yeast and moulds and common milk-borne pathogens namely Shiga toxin producing *Escherichia coli* (STEC), *Salmonella* spp., *Staphylococcus aureus, Streptococci, Brucella* spp. and *Mycobacteria*.

The microbiological analysis of milk is required to:

- ✓ Ensure the hygienic guarantee and the safety of consumers by allowing the detection of microorganisms and microbial toxins **(Guiraud, 1998).**
- ✓ Ensure the preservation of organoleptic and sensory characteristics of milk, thus extending its shelf life **(Vignola, 2002).**

The carried out analysis are made on:

- Total aerobic mesophilic flora.
- Total and fecal coliforms.
- Pathogenic microorganisms: *Staphylococcus aureus, Salmonella*

II.5.1. Enumeration method of microorganisms

There is as yet no perfect detection or enumeration method. Each has advantages and drawbacks, which require the user to have a clear understanding of the limitations, as well as the potential, of a particular technique.

- ✓ Homogenization:
  - Through a manual agitation.
- ✓ Preparation of dilutions:
  - We proceed to the dilution of samples due to the load contained microbial in milk and its difficulty of direct enumeration.
  - All manipulations are done in a sterile area near the Bunsen burner or in a microbiological host.

Method of operation

The dilutions are carried out in a classical way:

- Using a sterile Pasteur pipette, take, near the Bunsen burner, 1 ml of the sample to be analysed (raw milk) in a tube containing 9 ml of BPW and shake well the microbial suspension obtained (a dilution to 10^{-1}).
- From the first dilution (10^{-1}), transfer 1 ml to a tube containing 9 ml of diluent; BPW and shake the solution well (10^{-2} dilution).
- Repeat these steps until the (10^{-6}) dilution.

❖ **The enumeration of the colonies**

The colony enumeration is performed according to the following formula:

$$N=\sum c/ (n1+0.1n2)\ d$$

∑c: The sum of colonies in all boxes.

d: The dilution factor from which the first counts were obtained or the first positive dilution.

n1: Number of positive plates of the first dilution.

n2: Number of positive plates of the second dilution

II.5.2. Enumeration of the total aerobic mesophilic flora

This enumeration reflects the general microbiological quality of the milk. It concerns all the aerobic and aeroanaerobic microorganisms which develop on non-selective nutrient agar at 30°C for 3 days of incubation, likely to give visible colonies after 3 days of incubation at 30°C. It can degrade the food and thereafter digestive disorders (food poisoning or intoxication) or allergies to consumers.

The used technique is the counting in solid medium in Petri plate with mass plating on PCA medium **(Guiraud, 1998).**

Method of operation

- Prepare the sterile petri dishes.
- Inoculate the plates with 1 ml of each dilution (10^{-4}, 10^{-5} and 10^{-6})
- Add 15 ml of melted PCA (Plate Count Agar) cooled at 45 ± 1°C
- To homogenize the inoculum in the agar, make circular and back and forth movements.
- Allow it to solidify, then incubate it at 30°C for 72 h.

Figure 8: Preparation of PCA

- Expression of results

We count only petri dishes whose number of colonies are between 30 and 300. The result found is multiplied by the inverse of the dilution and then expressed in the number of CFU/ml **(Guiraud and Galzy, 1980).**

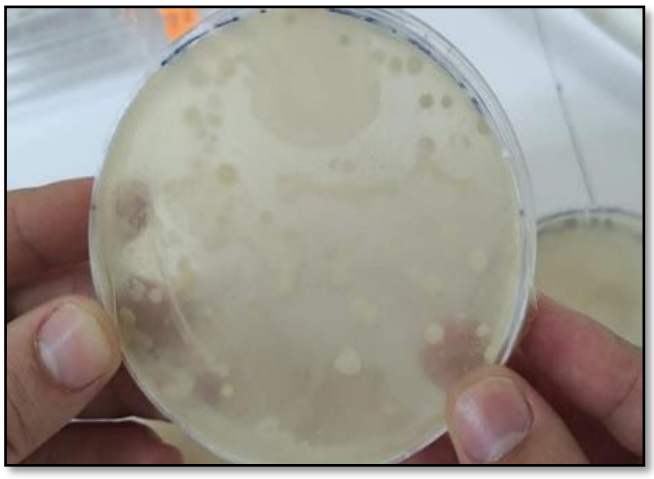


Figure 9: Colonies of TAMF.

II.5.3. Research for total and fecal coliforms

Colimetry is the set of methods allowing the research and the enumeration of coliforms, which indicates a fecal contamination.

Identification criteria of these anaerobic bacteria are production of gas from glucose (and other sugars) and fermentation of lactose to acid and gas within 48h at 35ºC **(Hitchins *et al.*, 1998).**

- **Total coliforms**

Total coliforms are gram-negative, aero-anaerobic, non-spore forming rods. **(Guiraud and Galzy, 1980).**

- **Fecal coliforms**

Fecal coliforms are coliforms that ferment lactose in EC medium with gas production within 48h at 44.5ºC **(APHA, 1970; Greenberg and Hunt, 1985; Paille *et al.*, 1987).**

Method of operation

- From each decimal dilution, inoculate a series of 12 tubes (with Durham bell) of 9 ml of BCPL in double concentration with 1ml of sample (for each dilution two tubes of BCPL).
- Incubate the tubes series at 37°C for (18 to 24) hours.
- From a BCPL (+) tube, inoculate by 1ml a tube containing 9 ml indole-free peptone water plus Durham bell.

- Incubation at 44°C for 24h.
- After incubation, add 2 to 3 drops of Kovacs reagent to the tube as a confirmation test.

II.5.4. Search for *Staphylococcus*

Staphylococcus aureus is the most commonly isolated contagious organism from mastitis bovine mammary glands worldwide **(Boss *et al.*, 2011).** The pathogen causes predominantly subclinical IMI **(Radostits *et al.*, 2007),** resulting in increased SCC and decreased milk production **(Blowey and Edmondson, 2010).**

The enumeration is performed on Chapman medium with streaking of 1ml of milk taken from the stock solution and the incubation is at 30°C for 24 hours.

Method of operation

- Prepare a sterile Petri dish.
- Add Chapman mannite agar.
- After solidification, take a drop of the raw milk with the platinum loop.
- Inoculate the drop by cross streaking and incubate at 37°C for 24 to 48h.
- The presence of *Staphylococcus aureus* is confirmed with the catalase test or GRAM stain test or coagulase test (using rabbit plasma).

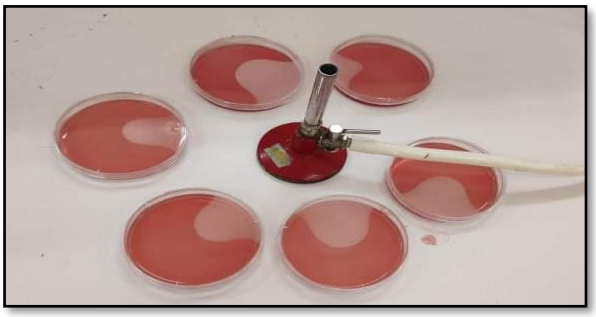


Figure 10: Preparation of Chapman mannite agar.

- Expression of results

The *Staphylococcus aureus* forms bulging colonies, shiny and more or less pigmented in yellow (**Guiraud and Rosec, 2004).**

✓ **Confirmation test**

Is performed with gram stain test, catalase test and Oxidase test.

II.5.5. Search and enumeration of *Salmonella*

Bacteria of the genus *Salmonella* belong to the *Enterobacteriaceae*, they are gram-*Bacilli*, facultative aero-anaerobes, and they reduce nitrates to nitrites. The toxic infections caused by *Salmonella* are the result of errors that allow an important bacterial growth.

Method of operation

1- Enrichment

- Prepare the medium SCB (Selenite - Cysteine broth);
- Pour 10ml of the dilutions in 100ml of SCB medium;
- Incubate at 37°C for 24h.

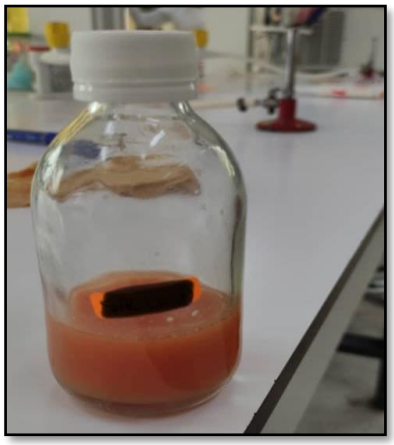


Figure 11: SCB result.

2- Isolation

- Isolate and inoculate on SS medium (*Salmonella-Shigella*), using the surface spreading technique.
- Incubate for 24h at 37°C.

Figure 12: Preparation of SS medium.

- Expression of results

Salmonella colonies appear as transparent colonies with a black center and absence of transfer of the color of the culture medium.

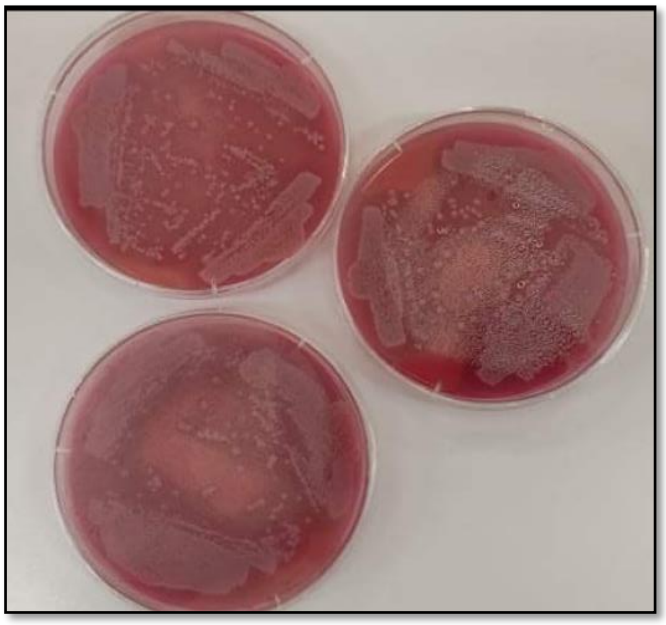


Figure 13: *Salmonella* colonies.

✓ **Confirmation test**

Is performed with gram stain test, catalase test, Oxidase test and the API 20E gallery test (Identification of gram-negative *Enterobacteria bacilli*).

II.5.6. Search for *Lactic Streptococci*

Is performed with M17 agar as an elective medium for the detection and differentiation of *lactic Streptococci.*

Method of operation

- Melt the M17 agar at 100°C in a water bath and maintain it at 45°C;
- Prepare a set of 6 Petri dishes for (10^{-4}, 10^{-5} and 10^{-6}) dilutions for each sample;
- Add 1ml of each dilution in the Petri dishes then pour 15ml of the agar;
- Homogenize the agar with the inoculum by making circular movements and let it cool down;
- Incubate the inverted plates at 45°C for 72h.

- Expression of results

- The resulted colonies appear as small and transparent (not very opaque) lenticular colonies.

✓ **Confirmation test**

Is performed with Gram stain test, catalase test, and the API 20Strep test (Identification system for *Streptococcaceae* and related organisms).

II.5.7. Confirmation tests protocol

1. The catalase test

The catalase test is a biochemical test for aerobic organisms that detects the production of catalase enzymes in the organism.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

There are many applications and method variations of the catalase test. These include the slide or drop catalase test and the tube method.

➢ **Slide method**

- Use a loop or a sterile wooden stick to transfer a small amount of colony growth to the surface of a clean, dry slide.
- Place a drop of 3% H_2O_2 in a glass slide.
- Watch the evolution of oxygen bubbles.

- Expression of results

- Immediate effervescence (bubble formation) ➔ catalase +
- No effervescence (no bubble formation) ➔ catalase –

2. Gram stain test

- **Technique**

➢ **Make a smear**

- Clean a microscope slide with alcohol (ethanol)
- Put a drop of H_2O on the slide
- Take a colony using a sterile platinum loop

- Rub the tip in the drop of water let it dry in the air
- Pass the slide 3 times in the small flame of the Bunsen burner to fix the sample with heat.

➢ **Coloration**

- Color the smear with a few drops of gentian violet for 2 minutes
- Discard the dye excess and rinse the slide very carefully by dripping the H_2O on the slide above the smear (not directly on the smear)
- Drop a few drops of lugol 2x on the smear for 45 seconds to 1 minute
- Discard the lugol excess and rinse the slide with H_2O_2
- Discolour the slide with alcohol 90° for 30 seconds
- Rinse it with H_2O
- Counter-coloring with 1/10 diluted fuchsine for 2 minutes (to visualize the Gram negative)
- Rinse it with H_2O_2 and let it dry in the air
- Observe it at immersion x100

- Expression of results

Cells → Gram + ➔ will appear in purple color
Cells → Gram - ➔ will appear in pink color
(Bouix et Leveau, 1991).

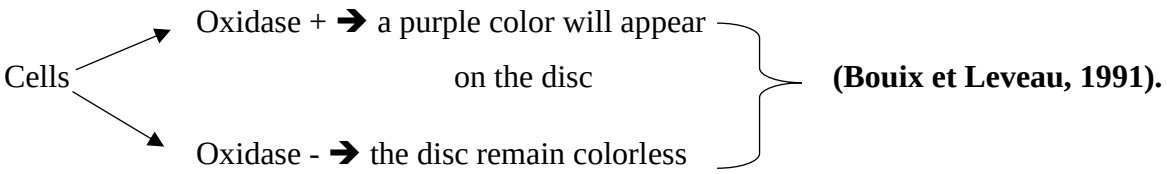
3. Oxidase test

Oxidase is an enzyme that reacts directly with oxygen, transferring electrons to it.

- **Technique**

- Take sterile ready-to-use oxidase discs;
- Place the discs on a sterile glass slide;
- Soak the disc with a drop of sterile physiological water;
- Place a fragment of a colony on top using a sterile Pasteur pipette.

- Expression of results



(Bouix et Leveau, 1991).

- **The API 20 gallery tests**

The well-established method for manual microorganism identification to the species level, bioMérieux's API identification products are test kits for identification of gram positive and gram negative bacteria and yeast. In our study we used the following API 20 tests: API 20E, API 20NE, and API 20Strep.

Results and discussion

Results and discussion

In our study, we collected two samples of raw milk from two different regions of Guelma province. The collection is performed under sterile conditions and in 250 ml vials. After the collection is complete, the milks are sent directly to the laboratory in a glacier in order to avoid possible fluctuations in the microbial flora.

- Firstly we carried out a measurement of the following physicochemical parameters: pH, dornic acidity, density, TDMC, DDM, boiling test.
- Secondly, we counted the following microbial groups: total aerobic mesophilic flora, total coliforms, faecal coliforms, lactic acid bacteria. A search is also carried out for the following pathogenic bacteria: *Staphylococcus aureus* and *Salmonella.*

I. Physico-chemical analysis

I.1. Evaluation of the physico-chemical quality of raw milk

The physico-chemical parameters were measured by the "Milk analyser", according to the manufacturer's instructions.

- **Determination of stability**

The results of the boiling test obtained for both samples of the analysed milk are positive, therefore all milk intended for consumption must be stable at boiling **(JORA N°35, 1998).**

Table 7: Results of physico-chemical analysis.

Samples / Parameters	Sample 1	Sample 2
pH	6.70	6.99
C (µS cm-1)	5.27	4.68
D (%)	30.60	20.88
T (°C)	19.40	20.50
FP (°C)	-0.551	-0.623
TA (°D)	18.00	22.20

C: Conductivity; **D:** Density; **T°:** Temperature; **FP:** Freezing point; **TA:** Titratable acidity;

- **pH**

The pH is a good indicator of how fresh the milk is **(Luquet, 1985).** The average pH is within the range [6.4 - 6.9] set by the **FAO**, these values are also in agreement with those reported by several authors such as **(Remeuf *et al;* 2003; Bamouh; 2006).**

According to **Alias (1984),** the pH is not a constant value and may vary depending on the lactation cycle and under the influence of food. For the sample 1 the pH of cow's raw milk that we have found complies with indicated standards. Sample 2 have value above the required standard.

According to **Mathieu (1998)** the pH variabilities are linked to climate, lactation stage, availability food.

- **Conductivity**

Electrical conductivity of milk is mainly due to its soluble salt fraction. Lactose does not conduct current, and fat decreases conductivity. The contribution of proteins and peptides is of minor importance **(Lanzanova *et al.*, 1993).**

Typically EC in normal milk is between 4.5 and 5.82 μS cm-1 at 25°C. Electrical conductivity in milk increases with milk sample temperature **(Hamann and Zecconi, 1998),** so EC is expected to be somewhat higher when EC is measured at milking because milk temperature is about 38°C when it leaves the teat cistern.

The present study agree reasonably well with the results of **(Hamana *et al.*, 1992 and Kaptan *et al.*, 2011),** who found absolute EC values to be from 5.04 to 5.82 μS cm-1.

The Electrical conductivity measurements have been widely used in the agro-industry food, for example to detect water contamination, to monitor the microbial growth and metabolic activity (**Curda and Plockova, 1995).**

- **Density**

The density of milk is the summary results of the densities of its various components. It is dependent on the amount of dissolved or suspended matter, changes in chemical composition of the constituents, and variations in physical states of components. Thus, milk density is influenced by various factors such as temperature history of samples, biological differences of

milk, and processing of milk. Among the various constituents, the variation in fat content is known to be the main cause in milk density variation **(Oguntunde and Akintoye 1991; Walstra, 1984).** However, according to **Sherbon (1988),** variations in the composition of fat and in the proportions of lactose, proteins, and salts may influence the milk density much less than variations due to the physical state of fat.

- **Temperature**

According to **Walstra (1984)** milk samples spoiled after the temperature was maintained for 5 hours at 40°C, and 6 hours at temperatures of 28°C and 16°C.

Temperature is an important variable. High or low temperature can affect milk in different factors, density, fat, the volume-weight, SNF…

- **Freezing point**

The freezing point of milk is an important indicator of quality, this parameter is used to determine the addition of water to milk (**Roginski *et al.*, 2003).**

According to **Fox and McSweeney (1998)**, the majority of cows produce milk with a natural freezing point of -0.525 to -0.565°C, with an average of about -0.54°C.

There are many factors that can mask the addition of water to milk, according to some authors, the amount of sugar, salts and powdered milk lower the freezing point and mask added water, and bacterial spoilage too causes freezing point depression, thus masking adding water **(Meredith *et al.*, 2007).**

- **Titratable acidity**

Comply with the values reported in the **JORA (1998)** (15°D - 17°D) and studies reported by **(Amiot *et al.*, 2002).** Also complies with FIL-AFNOR standards which are set between 16°D and 18°D. other studies have reported that the Titratable acidity of a milk may vary between a limit greater than 10°D and less than 21.4°D the acidity depends on the content of casein, mineral salts and ions, hygienic conditions during milking, total microbial flora and its activity metabolic, milk handling **(Guiraud, 1998).** Changes in the acidity of milk may be related to changes in the proportions of salts and proteins. A special role is played by the level of phosphates soluble and citrate and Ca^{2+} ions. Some of the micellar calcium phosphate passes to the soluble phase, thus increasing the concentration of Ca^{2+} ions and disrupting the structure

of micelles, which considerably affects the acidity of milk **(Muchetti, *et al.*, 1994; Czerniewicz *et al.*, 2006).**

I.2. Evaluation of the nutritional value of raw milk

Table 8: Results of the nutritional value.

Samples / Parameters	Sample 1	Sample 2
F (%)	3.00	6.82
P (%)	3.24	1.48
SNF (%)	8.76	7.53
S (%)	0.71	0.68
L (%)	4.77	5.34
AW	00.00	00.00

F: Fat; **P:** Protein; **SNF:** Solids-not-fat; **S:** Salts; **L:** Lactose; **AW:** Added water;

- **Fat**

Nearly all the milk fat in milk is in separate small globules. The state of fat dispersion may influence crystallization rate and milk density. A slow crystallization rate was obtained in more finely dispersed fat **(Walstra and Beresteyn, 1975).**

The fat content of the raw milk produced by cow's ranges from about 3.3% up to 5%. It varies by breed, and by diet, and can also be altered by selective breeding and genetic modification **(Bauman and Griinari, 2003).**

For the first sample the result we get is under the standard while for the second sample the result is above the standard.

- **Protein**

The total protein percentage of milk is generally considered to be about **3.5**%, of which 94 to 95 percent is in the form of true protein **(Davies *et al.*, 1983; Jenness, 1985).**

The protein content value is attributed to the factor according to **(Debry, 2001)** Genetics, lactation stage and age, as well as the influence of feed and season. **(Agabriel, 2001),** also showed that genetic factors and seasons It also affects the fat and protein content of milk.

- **Solid non-fat (SNF)**

Solid non-fat content is an important nutritional parameter of raw milk, which has a significant effect on the quality of milk, therefore one of the best candidates to be analysed for milk quality assurance. For cow milk, TS is 12 per cent 3.5%F and 8.5% SNF **(Bassbasi *et al.*, 2014).**

SNF content is the entire residue left after the complete evaporation of water from milk

- **Salt**

The salt of milk constitutes a small part of milk (8-9 g.L (-1)); this fraction contains calcium, magnesium, sodium and potassium for the main cations and inorganic phosphate, citrate and chloride for the main anions. In milk, these ions are more or less associated between themselves and with proteins.

- **Lactose**

Cow's milk contains 4 or 5% lactose. Lactose, being water soluble, is associated with the whey portion of dairy foods.

The average of lactose concentration in bovine milk is about 4.8%

- **Added water content**

No water were added for both samples the value is 00.00.

II. Microbiological analysis

II.1. Enumeration of total aerobic mesophilic flora

It is the most sought-after flora in microbiological analysis that informs us about the hygienic quality of raw milk.

The total aerobic mesophilic flora encompasses all saprophytic germs that can include pathogenic germs. They multiply very quickly in the open air and at temperatures between 20 and 40°C. The results obtained are given by table 9

Table 9: TAMF count results (UFC/ml) for the two different raw milk samples of the two regions

Samples	**Regions**	results (UFC/ml)	Log (UFC/ml)
01	Ras El Agba	2.1×10^4	4
02	Bourwayah	8.8×10^5	5.9

In our work according to table 9, we found that the number of TAMF colonies in the 1st sample is equal to (2.1×10^4 UFC/ml), and the number of TAMF colonies in the 2nd sample is equal to (8.8×10^5 UFC/ml).

It is found that the number of TAMF recorded in the 1st sample does not exceed the total flora contamination threshold which is [10^5 UFC/ml] according to **(JORA N°35, 1998).**

And we also noticed that the number of TAMF in the 2nd sample is exceeded the threshold of contamination, with a more severity of (8.8×10^5 UFC/ml).

The obtained results revealed that the 1st sample of raw milk analysed is of good quality, and the 2nd tested sample is of poor quality in the light of the standard established by **(JORA N°35, 1998)** which is [10^5 UFC/ml] and despite the temperature of the samples is almost relatively ambient varied between 20-24°C. The poor result of the 2nd sample proves that good hygienic practices are not respected during milking (cleaning hands, udder and bottle).

II.2. Enumeration of total and faecal coliforms

Coliform bacteria represent a very important group of contaminants in milk and dairy products, they are excellent indicators of old or recent fecal contamination.

II.2.1. Enumeration of total coliforms

The obtained results are shown in table 10 and table 11

Table 10: Total coliforms count results (UFC/ml) for the 1st raw milk sample of Ras El Agba region

Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Results	- -	+ +	- -	- -	+ -	- -
Number equal to the sum of the positive tubes	0	2	0	0	1	0
Regrouping	020 ◄►200 ◄►001 ◄►010					

Table 11: Total coliforms count results (UFC/ml) for the 2nd raw milk sample of Bourwayah region

Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Results	+ +	+ +	+ +	+ +	- +	+ +
Number equal to the sum of the positive tubes	2	2	2	2	1	2
Regrouping	222 ↔ 222 ↔ 221 ↔ 212					

According to table 10 and table 11 and by the method of counting microorganisms in liquid medium called the most probable number NPP (Mac Grady table), we find that the number of total coliforms in the 1st sample is equal to (0.92×10^2 UFC/ml), based on **(JORA Nº35, 1998)**, this value does not exceed the contamination threshold of [10^3 UFC/ml], and the number of total coliforms in the 2nd sample is equal to (1.5×10^4 UFC/ml) this value exceed the contamination threshold of [10^3 UFC/ml].

The poor result of the 2nd sample shows that contamination would be due to the lack of hygiene of the litter and the feeding behavior of dairy cows.

According to **(Magnusson *et al.*, 2007)**, heavily soiled litter contains more coliform bacteria and the incidence of mastitis increases in this case, suggesting greater contamination of the udder and milk. Other sources of contamination, such as poor transport conditions and poor milking hygiene, also need to be considered.

II.2.2. Enumeration of faecal coliforms

The obtained results are shown in table 12 and table 13

Table 12: Faecal coliforms count results (UFC/ml) for the 1st raw milk sample of Ras El Agba region

Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Results	- -	+ +	- -	- -	- -	- -
Number equal to the sum of the positive tubes	0	2	0	0	0	0
Regrouping	020 ↔ 200 ↔ 000 ↔ 000					

Table 13: Faecal coliforms count results (UFC/ml) for the 2nd raw milk sample of Bourwayah region

Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Results	- -	++	+ +	+ +	- -	+ +
Number equal to the sum of the positive tubes	0	2	2	2	0	2
Regrouping	022 ◄► 222 ◄► 220 ◄► 202					

According to table 12 and table 13 and by the method of counting microorganisms in liquid medium called the most probable number NPP (Mac Grady table), we find that the enumeration of fecal coliforms in the 1st analysed sample is equal to (0.92×10^2 UFC/ml), based on **(JORA N°35, 1998)**, this value does not exceed the contamination threshold of [10^3 UFC/ml], and the enumeration of fecal coliforms in the 2nd analysed sample is equal to (2×10^4 UFC/ml) its poor result (2nd sample) would be due to poor hygiene control as well as poor manipulation **(Guiraud et Rosec, 2004).**

From the results we obtained in the laboratory and according to **(Rozier *et al.*, 2006).** cited by (**Bouchibi and Boulame, 1997),** the fecal coliforms are *Escherichia coli.* **(Van Kessel *et al.*, 2004)** proved that coliform bacteria of the genus Escherichia are most common in the feces of dairy cows. They contaminate milk directly (by direct contact with the udder), or multiply during improper cleaning in the rinses of dairy utensils.

II.3. Search for *Staphylococcus*

Pathogenic germs such as *Staphylococcus aureus* are among the most dangerous germs in food products. Their presence causes a marked deterioration in the sanitary quality of the product and causes serious risks for the consumer. The obtained results are shown in table 14

Table 14: *Staphylococcus* search results (UFC/ml) for the two different raw milk samples of the two regions

Samples	**Regions**	***Staphylococcus***
01	Ras El Agba	-
02	Bourwayah	+

Based on the table results we noticed the absence of *Staphylococcus* colonies for the milk collected at the region of Ras El Agba, this result is confirmed at the standard (UFC/ml) **(JORA N°35, 1998)** which is (absence) of germs in raw milk. This absence can be explained by the good hygiene at the time of collection as well as the good health of the cow (the udder).

While we recorded a suspected presence of *Staphylococcus aureus* (yellow) colonies and *Staphylococcus epidermidis* (white) colonies for the milk collected at the region of Bourwayah, which is against the standard (UFC/ml) **(JORA N°35, 1998).**

Therefore, we made group of tests to determine which species of *Staphylococci* we resulted exactly.

Starting with oxidase test, all the analysed strains of our milk sample were found to be negative.

Figure 14: Oxidase test results.

Then we did a catalase test, all the strains analysed from our milk sample confirmed to be positive through the appearance of air bubbles

Figure 15: Yellow colony catalase test result

Figure 16: White colony catalase test result

Next, Gram stain test: we observed under the microscope gram-positive cocci bacteria in chain and grape clusters.

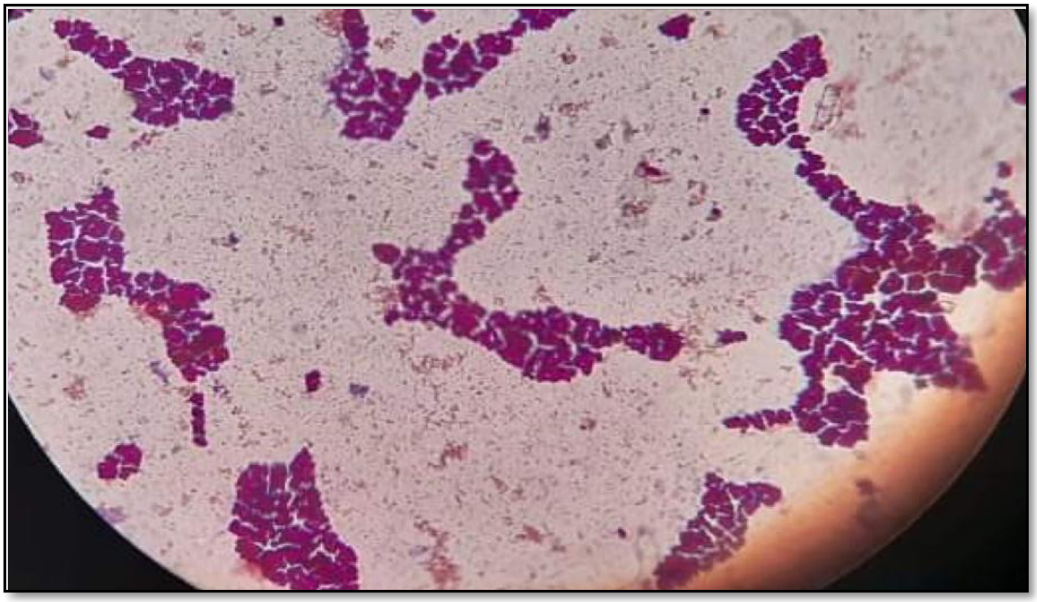


Figure 17: Gram stain result microscopic aspect.

Due to the lack of STAPH API gallery in our university, its presence couldn't be 100% identified to confirm our results, instead we did an API 20NE gallery test (Identification of gram-negative non-enterobacteria bacilli) on [https://lab.upbm.org/identifieur/galerie.php] to identify the other existing bacteria alongside the *Staphylococcus*.

The obtained results are shown in figures.

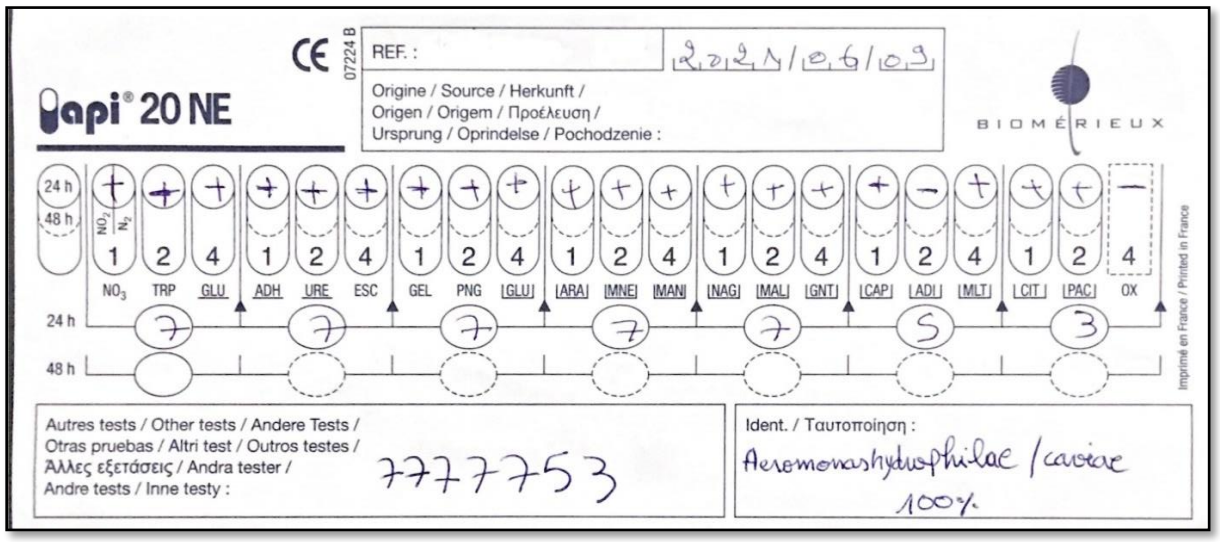


Figure 18: API 20NE result.

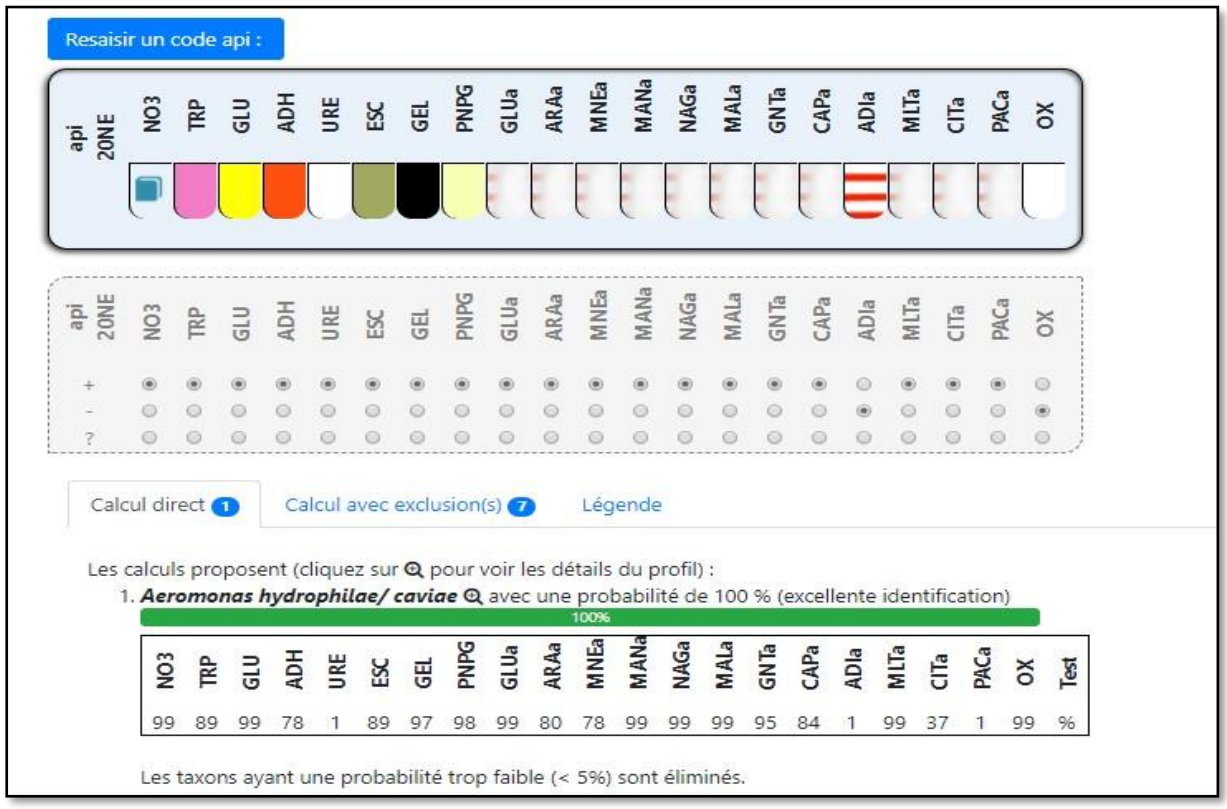


Figure 19: Identification of the different bacterial strains by the API 20NE gallery.

According to the figure we recorded the presence of *Aeromonas hydrophila*/*caviae* which is an organism commonly found in water, particularly during the warm season, though less commonly isolated as a pathogen in domestic animals and humans.

Based on our tests the existence *Staphylococcus aureus* in raw milk can be explained by udder infection, which is the main source of contamination, it is accompanied by an increase in the permeability between the blood compartment and the milk resulting in changes in the composition of the milk **(Rainard and Poutrel, 1993)** and also according to **(Thieulon, 2005)** contamination caused by infections of the udder, its first jets are heavily contaminated, hence the need to get rid of them; human skin, especially in the case of lesions; as well as the respiratory tract in case of infection (Angina pectoris) and contamination of the litter.

II.4. Search for *Salmonella*

The presence of this pathogenic microbial group in food products such as milk leads to great risks on the consumers and causes a clear degradation of the hygienic quality of the product. The results of our analysis of *Salmonella* search are shown in table 17

Table 15: *Salmonella* search results (UFC/ml) for the two different raw milk samples of the two regions

Samples	Regions	*Salmonella*
01	Ras El Agba	-
02	Bourwayah	+

According to table 15, the Microbiological analysis of this pathogenic microbial group showed an absence of contamination for the milk collected from Ras El Agba region, which comply with the **(JORA Nº35, 1998)** standards, it is in accordance with the ones found in Morocco by **(Srairi and Hamama, 2006),** and **(Haeghebaert *et al.*, 2003).** Overall, according to **(Haeghebaert *et al.*, 2003)** the isolation of *Salmonella* from raw milk is difficult to identify. While we recorded a total presence of contamination for the milk sample collected from Bourwayah region, which does not comply with the Algerian regulations.

Figure 20: Representative photo of colonies after incubation of both samples.

Oxidase test: all the analysed strains of our milk sample were found to be negative.

Figure 21: Oxidase test results of both samples.

Catalase test: all the strains analysed from our milk sample confirmed to be positive through the appearance of air bubbles.

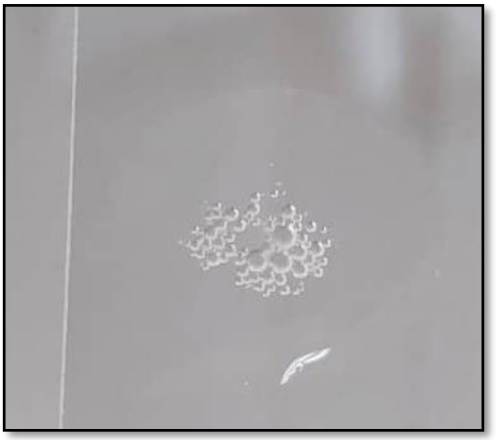


Figure 22: Catalase test result.

Gram stain test: we observed under the microscope gram-negative (pink color) *Bacilli* bacteria.

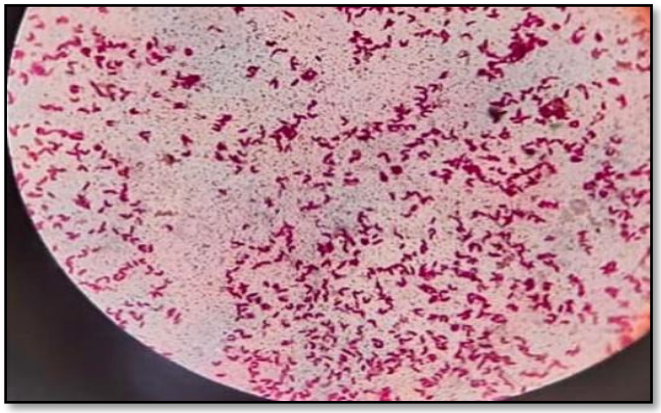


Figure 23: Gram stain result microscopic aspect.

Even though all the three tests above shows that the suspected bacteria is *Salmonella* but we can't confirm the results only through the API 20E gallery test (Identification of gram-negative *Enterobacteria Bacilli*) on [https://lab.upbm.org/identifieur/galerie.php].

The obtained result is shown in figures.

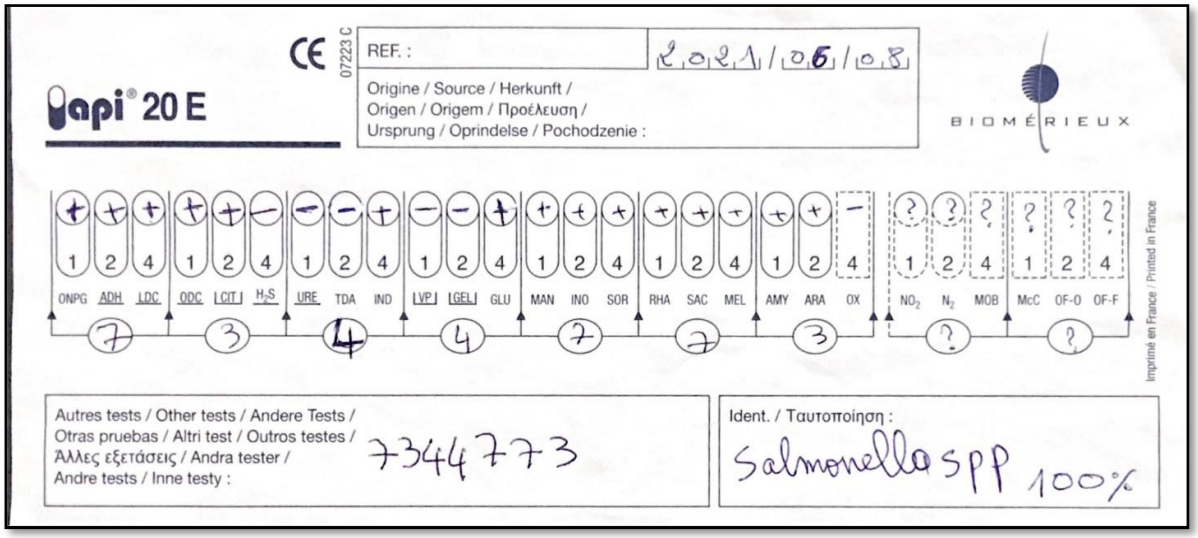


Figure 24: API 20E test result.

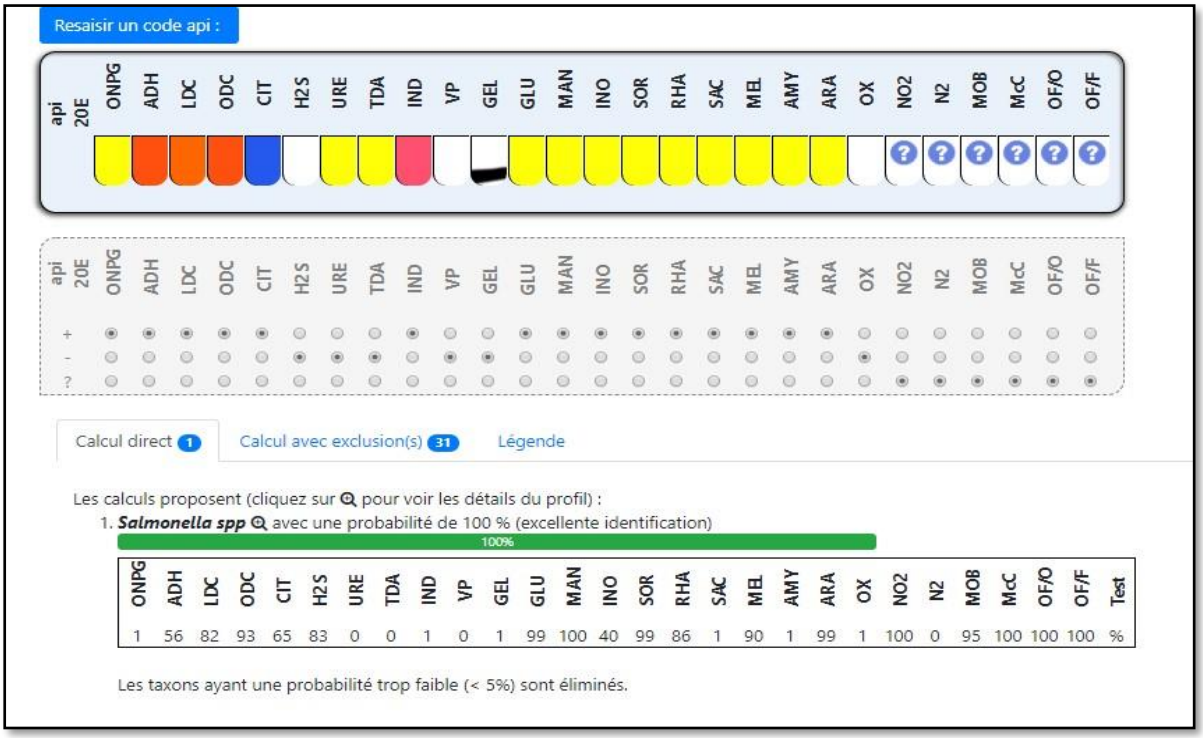


Figure 25: Identification of the bacterial strains by API 20E gallery.

According to **(Guy, 2006**), The main source of contamination in raw milk would be the excretion of *Salmonella* in the faeces, the spread of bacteria into the environment, then contamination of the skin of the udder and milking equipment, and finally into the milk.

II.5. Enumeration of *Streptococcus*

The count was carried out on a selective medium for *Streptococcus thermophilus* and *Lactococcus* (in particular: *Lactococcus lactis*) named M17. The obtained results are shown in table 16.

Table 16: Enumeration results (UFC/ml) for the two different raw milk samples of the two regions.

Samples	Regions	M17 (UFC/ml)	Log (UFC/ml)
01	Ras El Agba	Abs	0
02	Bourwayah	183.7×10^4	6.3

The Microbiological analysis results showed an absence of contamination for the milk collected from Ras El Agba region, which comply with the Algerian regulation standards.

Whereas we registered a contamination for the milk collected from Bourwayah region and found that the number of the appeared colonies is equal to (183.7×10^4 UFC/ml).

Thus we made group of tests to identify and confirm which kind of bacteria we resulted exactly.

Catalase test: the test was negative.

Figure 26: Catalase test result.

Oxidase test: the test result was negative.

Figure 27: Oxidase test result.

Gram stain test: we observed under the microscope gram-positive *Cocci* occurring in chains.

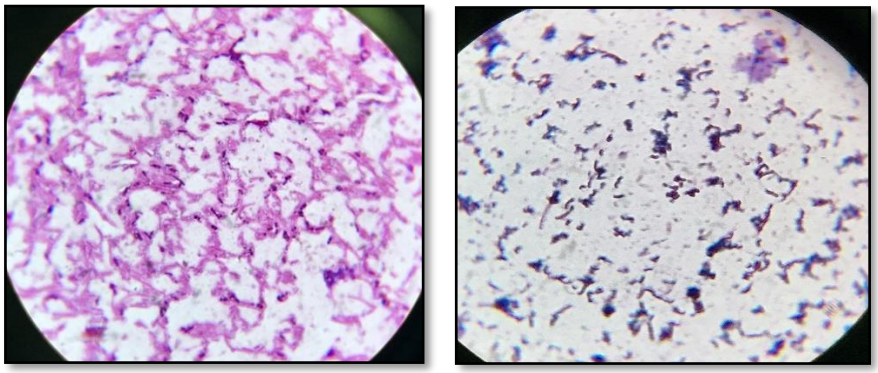


Figure 28: Gram stain test results microscopic aspect.

To finish with and to confirm our suspicion we did an API 20Strep test which is an Identification system for *Streptococcaceae* and related organisms on [https://lab.upbm.org/identifieur/galerie.php]. The obtained result is shown in figures.

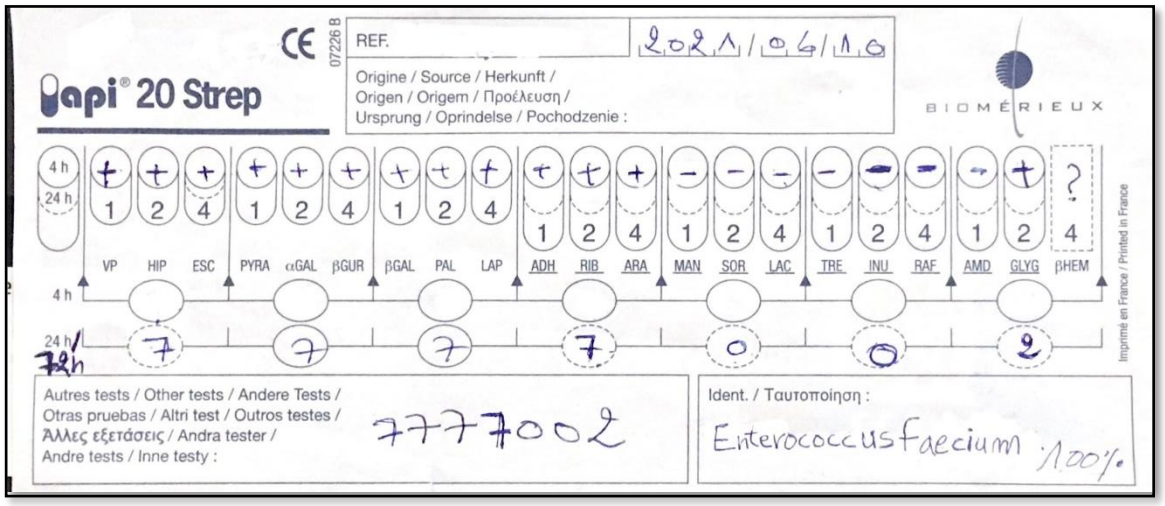


Figure 29: API 20Strep test result.

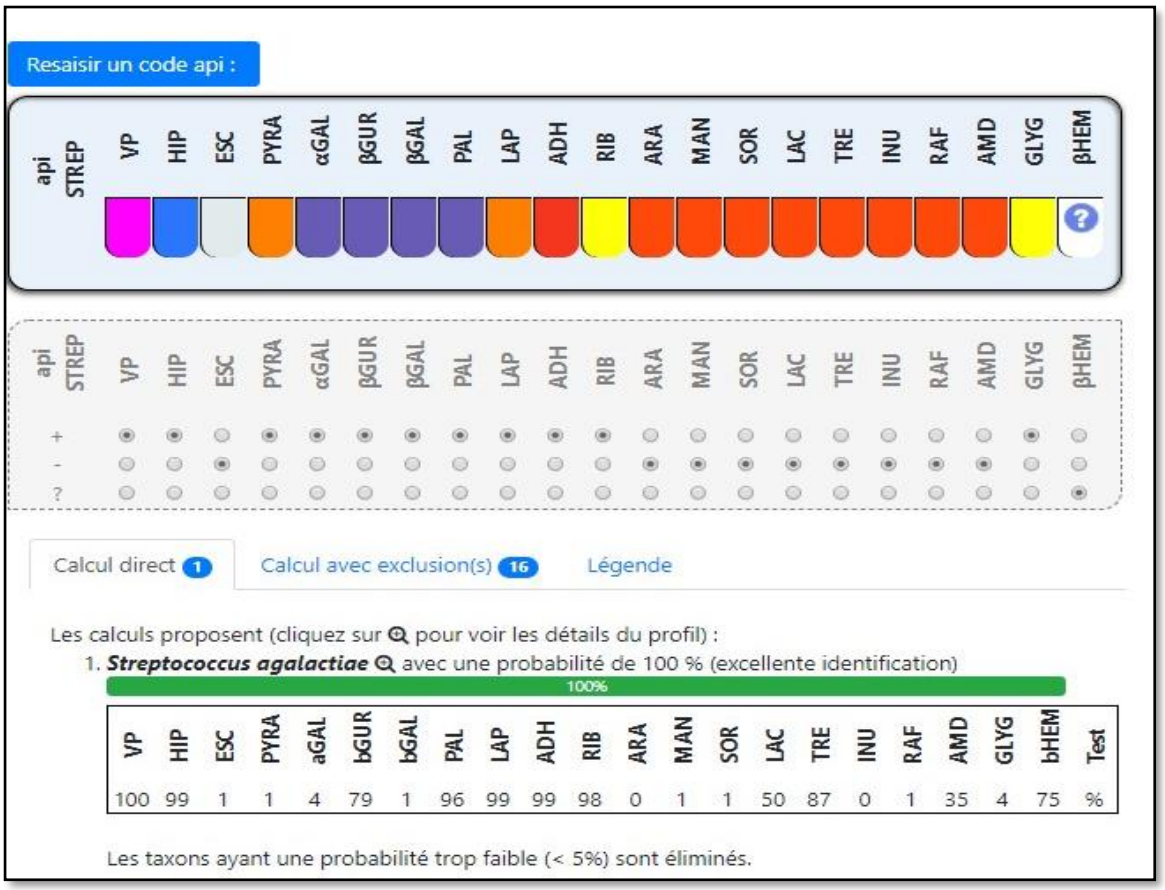


Figure 30: Identification of the bacterial strains by API 20Strep.

According to the API 20Strep result we confirmed that the suspected bacteria is *Enterococcus faecium* with a probability of 100%. According to **(Jett *et al.*, 1994; Ben Omar *et al.*, 2004)** in order to become pathogenic, *Enterococci* must exhibit virulence features associated with adhesion, translocation, and loss of immune response. It allows colonization and invasion of tissues and permeabilization of epithelial cells, thereby bypassing the host's immune defences **(Franz *et al.*, 2004)**. E. *faecium* is related with only 5-10% of infections stated by **(Kayser, 2003; Sánchez *et al.*, 2007),** it also has an antibiotic resist.

Conclusion

Indeed, milk is the first protein intake of humans and the first complete natural food from a young age. It contains the basic nutrients necessary for the proper development of the human body. Overall, milk consists of four types of important constituents: lipids, consisting mainly of ordinary fats (triglycerides), proteins (casein, albumin and globulin), carbohydrates, mostly of lactose, and salts. The content of these nutrients is influenced by intrinsic factors (species, breed, age, lactation periods) and extrinsic factors (season, diet).

Through this study, the performance of physico-chemical analysis of raw milk such as pH, dornic acidity, MG, density, conductivity..., revealed results that conform to the standards for the 1st sample and unbalanced results that don't fit to the standards for the 2nd sample.

Whilst the microbiological analysis of raw milk (count and search of total mesophilic aerobic flora, total and fecal coliforms, lactic *Streptococci*, *Staphylococci*, *Salmonella*) revealed a significant difference between the two samples.

The 1st sample is of good hygienic quality while the 2nd sample is of poor hygienic quality which exceed the Algerian regulation established by **(JORA N°069, 1998).**

In the light of these results, the hygienic quality of the raw milk sample collected from RAS EL AGBA and its analysis are mentioned as "satisfactory", which indicates that the hygienic conditions and the manipulations during the collect are being respected. While, the sample from BOURWAYAH district shows a catastrophic and a poor hygienic quality due to lack of hygiene whether during the collect or the unclean litter and the bad feeding habits from the garbage containers.

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Appendices

Appendix 1: Chapman mannitol Agar

- Chemical composition of the culture medium :

The theoretical formula of this medium in g/L of distilled water is:

- Tryptone	10.0
- Meat peptic peptone	5.0
- Meat extract	1.0
- Mannitol	10.5
- Sodium chloride	75.0
- Phenol red	0.025
- Agar bacteriological agar	15.0
pH= 7.5 Storage from 2 to 8°C	

Appendix 2: Enumeration Agar (PCA)

- The chemical composition of the culture medium :

The theoretical formula of this medium in g/L of distilled water is:

- Tryptone	5.0
- Glucose	1.0
- Yeast extract	2.5
- Agar	15.0
pH=7.0 +/-0.2 Autoclave sterilization at 121 °C for 15 minutes.	

Appendix 3: BCP lactose broth

- the chemical composition of the culture medium :

The theoretical formula of this medium in g/L of distilled water is:

- Casein Peptone	7.0
- Lactose	5.0
- Extract of b	1.0
- Bromocresol Purple 1%	0.03
pH = 6.7 +/- 0.2 at 25°C	

Appendix 4: Selenite-Cystine Broth

- the chemical composition of the culture medium :

The theoretical formula of this medium in g/L of distilled water is:

- Tryptosis	5.0
- Lactose	4.0
- Disodium phosphate	10.0
- Sodium hydrogenoselenite	4.0
- L-cystine	0.01

Appendix 5: SS Agar (SS AGAR)

- the chemical composition of the culture medium :

The theoretical formula of this medium in g/L of distilled water is:

- Peptone	5.0
- Pancreatic digestion of meat	5.0
- Lactose	10.0
- Bile salts	4.2
- Neutral red	0.025
- Bright green	0.0003
- Sodium Citrate	10.0
- Sodium thiosulfate	8.5
- Ammoniacal ferric citrate	1.0
- Agar bacteriological agar	15.0
pH= 7.0 +/- 0.2 Storage at 25°C	

Appendix 6: Temponated peptone water (20g/l)

- the chemical composition of the culture medium :

The theoretical formula of this medium in g/L of distilled water is:

- Peptone	10.0
- Sodium chloride	5.0
- Disodium phosphate anhydrous	3.56
- Monopotassium phosphate	1.5
Autoclave sterilization at 121°C for 15 minutes.	

Appendix 7: M17 AGAR

- the chemical composition of the culture medium :

The theoretical formula of this medium in g / L of distilled water is:

- Tryptone	2.50
- Meat peptic peptone	2.50
- Papainic soy peptone	5.00
- Autolytic yeast extract	2.50
- Lactose	5.00
- meat extract	5.00
- Sodium Glycerophosphate	19.00
- Magnesium sulphate	0.25
- Ascorbic acid	0.50
- Agar bacteriological agar	15.00
pH= 7.1 +/- 0.2 Storage at 25°C	

Appendix 8: Indole-free peptonated water

- the chemical composition of the culture medium :

The theoretical formula of this medium in g / L of distilled water is:

- Meat peptone	10.0
- Tryptone	10.0
- Sodium chloride	5.0
pH = 7.2 +/-0.2 Autoclave sterilization at 118-121°C for 15 minutes.	

Appendix 9: Mc Grady 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)					

Appendix 10: API 20E

TABLEAU DE LECTURE DE LA GALERIE MINIATURISEE API 20E

Microtube	Substrat	Caractère recherché	Lecture directe ou indirecte (Test si nécessaire)	Résultat +	Résultat -
ONPG	Ortho-Nitro-Phényl-Galactoside	β-galactosidase	Lecture directe		
ADH LDC ODH	Arginine Lysine Ornithine	Arginine dihydrolase Lysine décarboxylase Ornithine décarboxylase	Lecture directe		
CIT	Citrate	Utilisation du citrate	Lecture directe		
H_2S	Thiosulfate de sodium	Production d'H2S	Lecture directe		
URE	Urée	Uréase	Lecture directe		
TDA	Tryptophane	Tryptophane désaminase	Lecture indirecte Test : ajouter 1 goutte de Perchlorure de Fer		
IND	Tryptophane	Production d'indole	Lecture indirecte Test : ajouter 1 goutte de réactif de Kovacs		
VP	Pyruvate de sodium	Production d'acétoïne	Lecture indirecte (Attendre 10 minutes) Test : ajouter 1 goutte de KOH et d'α-napthol		
GEL	Gélatine emprisonnant des particules de charbon	Gélatinase	Lecture directe		
GLU à ARA	Substrat carboné	Utilisation de substrat carboné	Lecture directe		
NO_2^- / N_2	Nitrates (NO3)	Nitrate réductase	Lecture indirecte dans la cupule GLU Test : ajouter 1 goutte de réactif de Griess Ajouter de la poudre zinc en cas de résultat négatif		

Appendix 11: API 20NE

TABLEAU DE LECTURE

TESTS	COMPOSANTS ACTIFS	QTE (mg/cup.)	REACTIONS/ENZYMES	RESULTATS	
				NEGATIF	POSITIF
NO_3	potassium nitrate	0,136	réduction des Nitrates en nitrites	NIT 1 + NIT 2 / 5 min incolore	 rose-rouge
			réduction desNitrates en azote	Zn / 5 min rose	 incolore
TRP	L-tryptophane	0,2	formation d'indole (TRyptoPhane)	JAMES / immédiat incolore vert pâle / jaune	 rose
GLU	D-glucose	1,92	fermentation (GLUcose)	bleu à vert	jaune
ADH	L-arginine	1,92	Arginine DiHydrolase	jaune	orange / rose / rouge
URE	urée	0,76	UREase	jaune	orange / rose / rouge
ESC	esculine citrate de fer	0,56 0,072	hydrolyse (β-glucosidase) (ESCuline)	jaune	gris / marron / noir
GEL	gélatine (origine bovine)	0,6	hydrolyse (protéase) (GELatine)	pas de diffusion du pigment	diffusion du pigment noir
PNPG	4-nitrophényl-βD-galactopyranoside	0,22	β-galactosidase (Para-NitroPhényl-ßD-Galactopyranosidase)	incolore	jaune
GLU	D-glucose	1,56	assimilation (GLUcose)	transparence	trouble
ARA	L-arabinose	1,4	assimilation (ARAbinose)	transparence	trouble
MNE	D-mannose	1,4	assimilation (MANnosE)	transparence	trouble
MAN	D-mannitol	1,36	assimilation (MANnitol)	transparence	trouble
NAG	N-acétyl-glucosamine	1,28	assimilation (N-Acétyl-Glucosamine)	transparence	trouble
MAL	D-maltose	1,4	assimilation (MALtose)	transparence	trouble
GNT	potassium gluconate	1,84	assimilation (potassium GluconaTe)	transparence	trouble
CAP	acide caprique	0,78	assimilation (acide CAPrique)	transparence	trouble
ADI	acide adipique	1,12	assimilation (acide ADIpique)	transparence	trouble
MLT	acide malique	1,56	assimilation (MaLaTe)	transparence	trouble
CIT	trisodium citrate	2,28	assimilation (trisodium CITrate)	transparence	trouble
PAC	acide phénylacétique	0,8	assimilation (acide PhénylACétique)	transparence	trouble
OX	(voir notice du test oxydase)	-	cytochrome-oxydase	(voir notice du test oxydase)	

Appendix 12: API 20 STREP

TABLEAU DE LECTURE

TESTS	COMPOSANTS ACTIFS	QTE (mg/cup.)	REACTIONS/ENZYMES	RESULTATS			
				NEGATIF		POSITIF	
VP	sodium pyruvate	1,9	production d'acétoïne (Voges Proskauer)	VP 1 + VP 2 / jusqu'à 10 min (3) Incolore		Rose-Rouge	
HIP	acide hippurique	0,4	hydrolyse (acide HIPpurique)	NIN / jusqu'à 10 min Incolore/Bleu pâle Gris-bleuté		Bleu foncé/Violet	
ESC	esculine citrate de fer	1,16 0,152	hydrolyse ß-glucosidase (ESCuline)	4 h Incolore Jaune pâle	24 h Incolore Jaune pâle Gris clair	4 h Noir Gris	24 h Noir
PYRA	acide pyroglutamique-ß-naphtylamide	0,0256	PYRrolidonyl Arylamidase	ZYM A + ZYM B / 10 min (PYRA à LAP) (1) au besoin décoloré par éclairement intense Incolore ou Orange très pâle		Orange	
αGAL	6-bromo-2-naphtyl-αD-galactopyranoside	0,0376	α-GALactosidase	Incolore		Violet	
ßGUR	acide naphtol-ASBI-glucuronique	0,0537	ß-GlUcuRonidase	Incolore		Bleu	
ßGAL	2-naphtyl-ßD-galactopyranoside	0,0306	ß-GALactosidase	Incolore ou Violet très pâle		Violet	
PAL	2-naphtyl phosphate	0,0244	Phosphatase ALcaline	Incolore ou Violet très pâle		Violet	
LAP	L-leucine-ß-naphtylamide	0,0256	Leucine AminoPeptidase	Incolore		Orange	
ADH	L-arginine	1,9	Arginine DiHydrolase	Jaune		Rouge	
				4 h	24 h	4 h	24 h
RIB	D-ribose	1,4	acidification (RIBose)	Rouge	Orange/ Rouge	Orange/ Jaune	Jaune
ARA	L-arabinose	1,4	acidification (ARAbinose)	Rouge	Orange/ Rouge	Orange/ Jaune	Jaune
MAN	D-mannitol	1,36	acidification (MANnitol)	Rouge	Orange/ Rouge	Orange/ Jaune	Jaune
SOR	D-sorbitol	1,36	acidification (SORbitol)	Rouge	Orange/ Rouge	Orange/ Jaune	Jaune
LAC	D-lactose (origine bovine)	1,4	acidification (LACtose)	Rouge	Orange/ Rouge	Orange/ Jaune	Jaune
TRE	D-tréhalose	1,32	acidification (TREhalose)	Rouge	Orange/ Rouge	Orange/ Jaune	Jaune
INU	inuline	5,12	acidification (INUline)	Rouge	Orange/ Rouge	Orange/ Jaune	Jaune
RAF	D-raffinose	3,12	acidification (RAFfinose)	Rouge	Orange/ Rouge	Orange/ Jaune	Jaune
AMD	amidon (2)	2,56	acidification (AMiDon)	Rouge	Orange/ Rouge	Orange/ Jaune	Jaune
GLYG	glycogène	1,28	acidification (GLYcoGène)	Rouge ou Orange		Jaune franc	

Appendix 13: JORA N°35, 1998

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ANNEXE I

CRITERES MICROBIOLOGIQUES RELATIFS A CERTAINES DENREES ALIMENTAIRES

TABLEAU I

CRITERES MICROBIOLOGIQUES DES LAITS ET DES PRODUITS LAITIERS

PRODUITS	n	c	m
1. **Lait cru :**			
— germes aérobies à 30° C	1	—	10^5
— coliformes fécaux	1	—	10^3
— streptocoques fécaux	1	—	abs/0,1ml
— *Staphylococcus aureus*	1	—	absence
— clostridium sulfito-réducteurs à 46° C	1	—	50
— antibiotiques	1	—	absence
2. **Lait pasteurisé conditionné :**			
— germes aérobies à 30° C	1	—	3.10^4
— coliformes :			
* sortie usine	1	—	1
* à la vente	1	—	10
— coliformes fécaux			
* sortie usine	1	—	absence
* à la vente	1	—	absence
— *Staphylococcus aureus*	1	—	1
— phosphatase	1	—	négatif
3. **Lait stérilisé et lait stérilisé UHT (nature et arômatisé) :**			
— germes aérobies à 30° C	5	2	< 10/0,1 ml
— test de stabilité	5	0	négatif
— test alcool	5	0	négatif
— test chaleur	5	0	négatif
4. **Lait concentré non sucré :**			
— test de stabilité	5	0	négatif
— test alcool	5	0	negatif
— test chaleur	5	0	négatif
5. **Lait concentré sucré :**			
— germes aérobies à 30° C	5	2	10^4
— coliformes	5	0	absence
— *Staphylococcus aureus*	5	0	absence
— clostridium sulfito-réducteurs à 46° C	5	0	absence
— levures et moisissures	5	0	absence
— *Salmonella*	5	0	absence
6. **Lait déshydraté conditionné (1) :**			
— germes aérobies à 30° C	5	2	5.10^4
— coliformes	5	2	5
— *Staphylococcus aureus*	5	0	absence
— clostridium sulfito-réducteurs à 46° C	5	0	absence
— levures et moisissures	5	2	50
— *Salmonella*	5	0	absence
— antibiotiques	1	0	absence

Milk is considered a complete and balanced food because of its richness in many nutrients (proteins, lipids, mineral salts, lactose and vitamins).

Our work is focused on determining the status of the physicochemical and microbiological quality of raw milk in two regions (RAS EL AGBA and BOURWAYAH) of the GUELMA province. The number of samples was set at two, taken from two farms in these regions. It has two parts: a bibliographic part and an experimental part.

The obtained results during this study indicate that the milk collected at RAS EL AGBA has an acceptable physico-chemical quality for all the parameters thus in accordance with the standards, and that the milk sample collected at BOURWAYAH region shows a differentiation between the parameters, some are unacceptable and some comply with the standards.

Hence, the analysed RAS EL AGBA raw milk appeared to be of acceptable microbiological and physicochemical quality which generally falls under a better hygiene practices, application of good and well moderated feeding, while the BOURWAYAH raw milk appeared to be of a really poor microbiological and physicochemical quality, it exceed the standards recommended by the (**JORA Nº35, 1998**) which is due to the lack of hygiene and the quality of the poor, improper and harmful feeding applications in farms.

- **Key words**: Raw milk, Microbiological analysis, Physico-chemical analysis, Hygiene, Quality, RAS EL AGBA, BOURWAYAH.

Le lait est considéré comme un aliment complet et équilibré en raison de sa richesse en nombreux nutriments (protéines, lipides, sels minéraux, lactoses et vitamines).

Notre travail est axé sur la détermination de l'état de la qualité physico-chimique et microbiologique du lait cru dans deux régions (RAS EL AGBA et BOURWAYAH) de la province de GUELMA. Le nombre d'échantillons a été fixé à deux, prélevés dans deux fermes de ces régions. Il comporte deux parties: une partie bibliographique et une partie expérimentale.

Les résultats obtenus au cours de cette étude indiquent que le lait collecté à RAS EL AGBA présente une qualité physico-chimique acceptable pour tous les paramètres ainsi conformes aux normes, et que l'échantillon de lait collecté dans la région de BOURWAYAH montre une différenciation entre les paramètres, certains sont inacceptables et certains respectent les normes.

Ainsi, le lait cru RAS EL AGBA analysé semblait être de qualité microbiologique et physico-chimique acceptable qui relève généralement des bonnes pratiques d'hygiène, une application alimentaire bonne, propre et bien modérée alors que le lait cru BOURWAYAH semblait être d'une qualité microbiologique et physico-chimique vraiment médiocre, il dépassait les normes recommandées par la **(JORA N°35, 1998)** qui est due au manque d'hygiène es applications alimentaires pauvres, inappropriées et nocives dans les fermes.

- **Mots clés :** Lait cru, Analyse microbiologiques, Analyse physico-chimiques, Hygiène, Qualité, RAS EL AGBA, BOURWAYAH.

الملخص

يعتبر الحليب طعاما كاملا ومتوازنا بسبب ثرائه في العديد من العناصر الغذائية (البروتينات والدهون والأملاح المعدنية واللاكتوز والفيتامينات).

يركز عملنا على تحديد حالة الجودة الفيزيائية والميكروبيولوجية للحليب الخام في منطقتين (رأس العقبة و بوروايح) في محافظة قالمة. تم اخذ عينة من الحليب الخام من كل منطقة، حيث تحتوي المذكرة على جزأين: جزء نظري وجزء تجريبي.

تشير النتائج التي تم الحصول عليها خلال هذه الدراسة إلى أن الحليب الذي تم جمعه من راس العقبة له جودة فيزيائية كيميائية مقبولة لجميع المعلمات وبالتالي تتوافق مع المعايير ,وأن عينة الحليب التي تم جمعها في منطقة بوروايح تظهر اختلاف بين المعلمات ، بعضها غير مقبول وبعضها يتوافق مع المعايير.

ويبدو أن حليب رأس العقبة الخام الذي تم تحليله ذو جودة ميكروبيولوجية وفيزيائية مقبولة والتي تندرج عموما تحت ممارسات النظافة و التغذية الجيدة و المنتظمة في حين يبدو أن حليب بوروايح الخام ذو جودة ميكروبيولوجية وفيزيائية سيئة جدا، فإنه يتجاوز المعايير الموصى بها من قبل **(JORA Nº35, 1998)** والذي يرجع إلى نقص النظافة و جودة التغذية الغير سليمة و المضرة للابقار.

- **الكلمات المفتاحية :** الحليب الخام ، التحليلات الميكروبيولوجية ، التحليلات الفيزيائية و الكيميائية ، النظافة ، الجودة ، رأس العقبة، بوروايح.