Journal of Fundamental and Applied Sciences

Research Article

ISSN 1112-9867

Available online at http://www.jfas.info

BIOCHEMICAL AND MICROBIOLOGICAL IDENTIFICATION OF COW'S MILK FOR THE MANUFACTURE OF SOFT CHEESE

F. Mesli*[1], A. Lazouni Hamadi[2]

[1]Laboratory of Naturals Products and Bioactives- LASNABIO, University of Tlemcen, B. P. 119, 13000 Tlemcen, Algeria

[2]Laboratory of Natural Products, Department of Biology, Faculty of Science, University of Tlemcen, B. P. 119, 13000 Tlemcen, Algeria

Received: 22 April 2019 / Accepted: 20 August 2019 / Published online: 01September 2019

ABSTRACT

Our work focuses on the study of the technological process of making a soft cheese; five samples were the subject of our investigations. These were analyzed during the period of high lactation (January to March 2017). Microbiological and biochemical tests were carried out on five microbial groups and pathogenic organisms. Biochemical identifications are performed to target the most likely bacteria. All the results have been compared is validated by contribution to experimental results as well as theoretical work show a compatibilities and validation by contribution to the norm. The microbiological analyzes of the five samples studied show the total absence of the seeds sought with the exception of FTAM, with a low presence not exceeding the threshold of acceptability. These results indicate that the samples of the milk used in the manufacture of soft cheese are very good from a microbiological point of view.

Keywords: Milk, Microbiology, Identification.

Author Correspondence, e-mail: meslifouzia2018@gmail.com

doi: http://dx.doi.org/10.4314/jfas.v11i3.12

1. INTRODUCTION

1.1. First Subtitle

The principal components of milk by ascending order according to [1] are: Water, glucids mainly them lipids, primarily of triglycerides gathered in fatty globules, rock salt, proteins,

caseins gathered out of micelles, soluble albumins and globulines, trace elementss but with the important biological role, enzymes, vitamins and trace elements. It is consisted 98.5% from glycérides (esters of fatty-acid and glycerol), polar phospholipide 1% and 0.5% of liposoluble substances cholesterol, hydrocarbons and vitamins A, D, E and K [2] that density oscillates between 1.028 and 1.034. It must be higher or equal to 1.028 with 20°C [3]**.** The importance and the nature of the bacteria contaminants milk, depend, of the medical condition of the animal, the nature of fodder [4] but also hygienic desconditions at the time of the draft, the collection, the handling and the temperature of conservation of milk.Milk, basic food of most of the Algerians, with 115 liters consumed per capita and a year. It is a complex food with the many virtues; it is the essential companion of a balanced food [5].

1.2 Second Subtitle

This wealth of the raw milk made of this one a medium favorable for the multiplication of the germs coming from the bad conditions of hygiene of the draft as has the medical condition of the animals. The pure and simple currency of the Safilait dairy is quality must take precedence over anything else.However, the characteristics of the development of the total quality (physical, chemical and hygienic) of this product and specificities of the context of bovine breeding in Algeria, should have imposed, well rather, the conduit of research tasks applied to these problems. It is given like objective to evaluate the microbiological degree of contamination of the raw material, the raw milk of mixture, intended for the manufacturing of standard soft cheese Camembert cheese (100% cow's milk), in optics to identify the failures upstream sector on the level of the farms. Our research will relate to the pilot germs of defect of hygiene: total flora, flora psychrotrophe, coliformes total, coliformes fecal, Escherichia coli and streptococci fecal, as well as the pathogenic germs: Salmonella spp., Staphylococcus aureus. It is in this context that this research task fits, whose main aim is to contribute to clarify this subject.

2. RESULTS AND DISCUSSION

2.1 Results of the microbiological analyses

2.1.1 Research and enumeration of the aerobic germs Mésophiles total

Table 1. The analyses microbiologies of the analyzed samples (FTAM)

Germ / Samples	FTAM /ml								
	10^{-1}			10^{-2}			10^{-3}		
	A 24h	A 48h	A 72h	A 24h	A 48h	A 72h	A 24h	A 48h	A 72 h
LV1	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
LV2	Abs	Abs	Abs	30	20	20	Abs	Abs	Abs
LV3	10	10	25	Abs	Abs	Abs	20	Abs	Abs
LV4	Abs	20	Abs	Abs	10	Abs	Abs	Abs	Abs
LV5	Abs	21	30	Abs	Abs	Abs	ABS	Abs	Abs
Normalizes	<106								

In the five samples one notes a microbial load total (FTAM) variable and normal. A microbial load definitely lower than the standards can be explained by lesbonnes practical hygiene at the time of the draft and the handling of milk, as well as the hygienic bonnesconditions of breeding and production. Therefore, we can conclude that the quality of the milk intended for the analysis is acceptable, ceséchantillons raw milk of cow intended for the manufacturing of cheese with paste molletype Camembertprésente a hygienic good quality. The improvement of the hygiene of the draft, the collection and the conservation rapideau cold would make it possible to reduce the microbial load [6].

Table 2. The analyses microbiologies of the samples analyses

Germ Samples	Coliforme	Salmonella	Streptococcifecal	Staphylococcus aureus
LV1	0.1	Abs	Abs	Abs
LV2	0.2	Abs	Abs	Abs
LV3	0,00	Abs	Abs	Abs
LV4	0.2	Abs	Abs	Abs
LV5	0.4	Abs	Abs	Abs
Normalizes	**$<3.10^4$**	**Abs**	**Abs/0.1ml**	**Abs**

Salmonellas The microbiological analysis of this pathogenic microbial group did not show contamination. The complete lack in all the samples meets the standards, which indicates that our milk is of microbiological good quality, hygienic and that the conditions of breeding, draft, transport, conservation and of storage are good conditions.

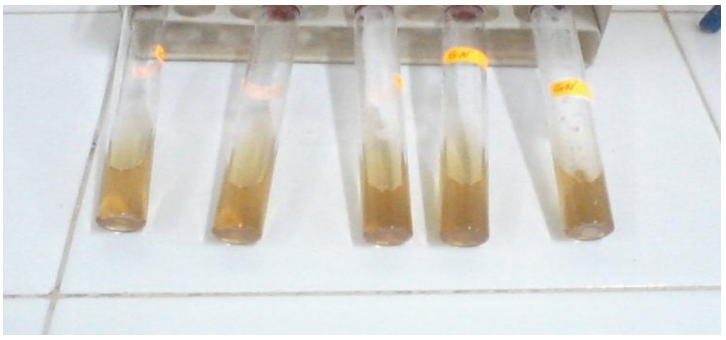
Coliformes The research and the enumeration of the fecal coliformes make it possible to appreciate the importance of contamination of milk and the dairy products (VIGNOLA, 2002). Mocquot and Guittonneau (1939) showed that the coliformes of the kind Escherichia are most frequent in the excrements of the dairy cows. They directly contaminate milk (by direct contact with worse), or multiply during a bad cleaning in the dishwaters of the dairy ustensils

Fecal Streptococci The complete lack in all the samples can be explained by the good practices of hygiene at the time of the draft and the handling of milk, as well as the hygienic good conditions of breeding and production.

Staphylococcus aureus The complete lack of these germs in milk can be explained by the good compliance with the rules of general hygiene. The research and the enumeration of the staphylococcus aureus are in keeping with the health status of the cows and the hygienic conditions of the draft.

2.2 Results of the biochemical identifications

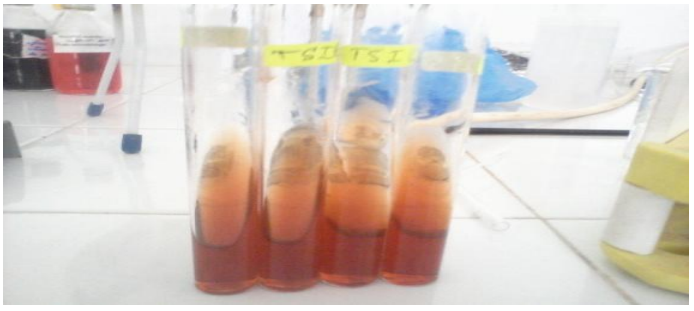
2.2.1 Conservation the colonies intended under investigation are sown on agar nutritive out of tube tilted. After 24 hours of incubation to 37 °C carried out the conservation with +4 °C. Two bacterial strains were identified: the salmonella and coliformes.



After the 24 hours incubation to 37 °C

Fig.1. Agar nutritive with solidification

2.2.2 Recherchede the fermentation of the glucoseC' is a degradation of the lactose production or not of gas and H2S: After checking of the stocks, a sowing on agar triples sugar Iron (TSI) is carried out by central puncture especially the depth of the tube and by scratches parallel at surface, after 24 hours of incubation to 37°c, according to degraded sugar one witnesses an acidification on various levels:



After the 24 hours incubation to 37 °C

Fig.2. Sowing on agar triples sugar Iron (TSI)

Results

The colonies characteristic of a positive TSI are mended on various mediums for the research of the essential biochemical characters:

- Eau peptone exempts indol
- Clark and Lubs
- Citrates of Simmons
- Mannitol and mobility.

2.2.2.1 Test Indol is made on medium water peptone (Indol). This medium is sown with the stock to test. Incubation is done with 37°c during 24 hours. The required characters it is tryptophan after addition of 2-3 drops of the reagent of kovacs.

Results: Formation of a red ring on the surface Indol +

Red absence of colouring Indol -

2.2.2.2 Test RM and VPSe made on medium Clark and Lubs. This last allows differentiates fermentations leading to mixed acids or butylene – glycolic. This medium is sown with the stock to test. Incubation is done at temperature optimal during 24/48 hours. The required characters it is the bacteria producing of the organic acids. The addition of 2-3 drops of the methyl RM red indicates the presence of mixed acids (acid formic, acetic and lactic)

The addition of the voges proskawer VP (pink – red) indicates the presence of butylene – glycolic.

Results: Red RM + /VP+ Yellow RM+/VP-

2.2.2.3 Test By the use of citrate: One uses the citrate medium of Simmons who allows the description of an enzyme known as (citrate perméase) which thanks to its action carried out the degradation of the citrate (only source of carbon). Citrate + $3H_2O$ $\longrightarrow$ acide citrique $+3OH^-$

Results: The positive reaction results in the blueing of the medium of to alkalisation Citrate +

In the contrary case, the citrate keeps its initial color Citrate -

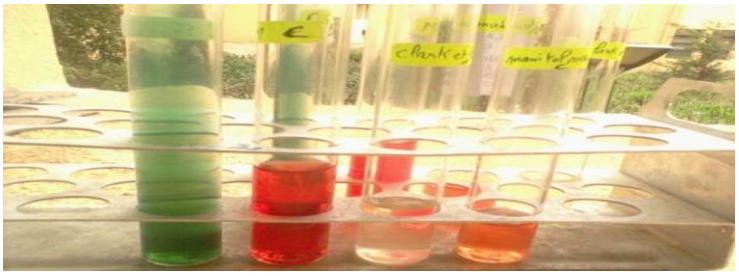
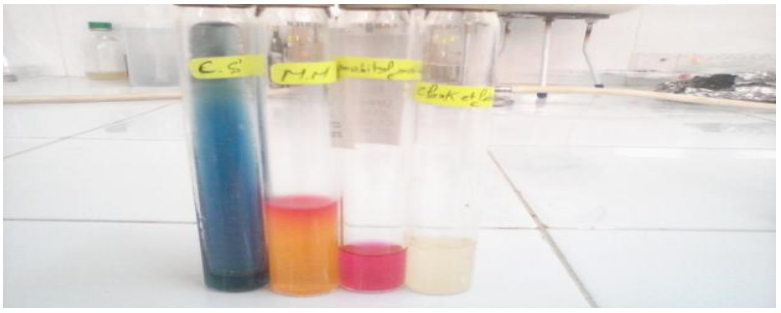


Fig.3. Sowing by the citrate medium of Simmons

2.2.2.4 Test By the use of the mannitol: This medium makes it possible simultaneously to seek the mobility and the use of the mannitol and to differentiate between Kélebssiella and enterobactery. The medium is sown by central puncture, after incubation with 37°C during 24 hours, the acidification of the medium represents of this fact the degradation of the mannitol.



After the 24 hours incubation to 37 °C

Fig.4. Sowing by the mannitol medium

Results: Mobility results in a horizontal diffusion starting from the line of sowing involving a disorder of the medium.

Lactose +: yellow slope because the use of lactose is supported by the conditions of aerobiosis.

Glucose +: yellow base Lactose +: E. coli – kélebssiella – Entérobactérie

Glucose +: E. coli – kélebssiella – Entérobactérie

2.2.3 Test ONPG It makes it possible to quickly detect the bacteria which ferment lactose. ONPG means: (O: ortho, NR: nitro, P: phenol, G: glacto – pyranoside). The technique used one prepares a dense suspension of 0.5 ml of water physiological added with a disc of ONPG and incubated with 37° during 24 hours. Required characters; a β – galactoside perméase membrane, a β - galactosidase

Results*:* The positivity of the test is revealed when the suspension takes a yellow color ONPG+. In the contrary case, the medium remains colourless ONPG-

2.2.4 Test oxydase: This test is at the base of identification of the Gram- bacteria

The technique used on a beforehand sterile blade, one places a disc at oxydase soaked with physiological water. The suspect colony, taken, is then deposited on the disc.

Required characters; enzyme phenylene diamine oxydase.

Results: The bacteria producing the oxydase, immediately give (1 min approximately) a purplished color. Oxydase+ The colourless disc oxydase

2.2. 5 Test of LDC, ODC and ASH

The test by three systems of lysin decarboxylase (LDC), ornithin decarboxylase (ODC), arginine dithydrolase (ASH). Décarboxylases LDC divide the amino-acids by involving the formation of the corresponding amine as well as the release of CO_2.

$$R - NH_2 - COOH \longrightarrow R - CH_2 - NH_2 + CO_2$$

Lysin décarboxylase acts on lysin by giving it (cadaverine). Ornithin décarboxylase ODC acts on ornithin involving the formation of (putrescine). In the same way the arginine dihydrolase ASH by action on similar gift substrate gives (the agimatine). The medium used (medium FALCOW) is sown by the stock to test. This last is added with a few drops of sterile oil of limestone to create the anaérobiose.

Results: A positive reaction results in an alkalisation of the medium like by a turn with the yellow

2.3 Identification of one Entérobactérie using an API system 20th

API 20th is a system for the identification of Entérobacteriacese and other Gram- bacilli using of the galleries. An API gallery 20th is a set of 20 micro tubes containing of the mediums in forms dehydrated by putting the realization of 23 biochemical tests.

Preparation of the gallery: To join together the bottom and lid of one limp of incubation and to set out again approximately 5 ml of water distilled in the cells to create a wet atmosphere.

To register the references of the stocks to be identified on the side strip of limps. To withdraw the gallery of its individual packing and to deposit it in limp of incubation.

Preparation of the bacterial suspension: In a tube containing 5 ml of sterile distilled water carried out a bacterial suspension by homogenizing the bacteria carefully takes starting from an agar for insulation of enterobactery.

Incubation of the gallery: For tests CIT, VP, FREEZING, to fill tubes and cups. For all the other tests to fill only the tube (the cup must remain empty). For tests ADH, LDC, ODC, and RUE, to create a anaérobiose by filling their cup with paraffin oil. To close limps it of incubation. To incubate 24 hours with 37

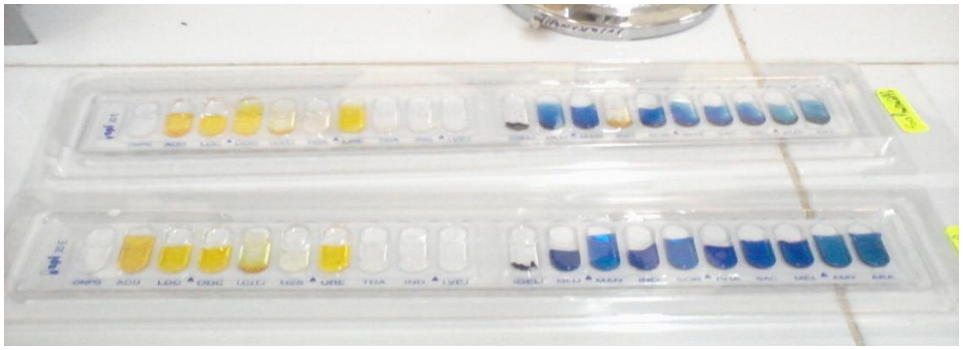
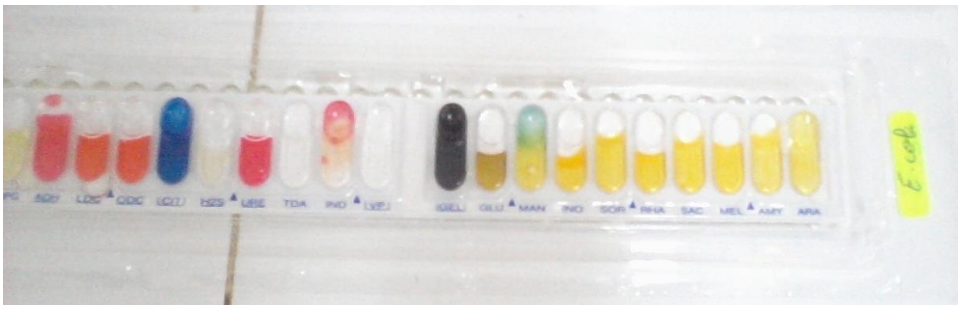
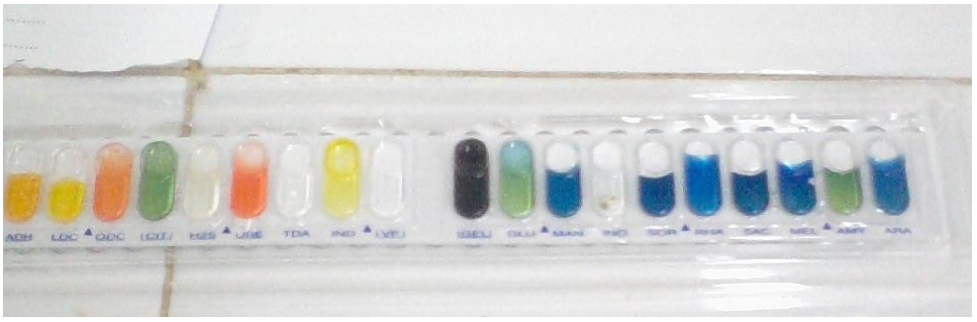


Fig.5. API plate 20th by Sowing of distilled water and it paraffin oil



After the 24 hours incubation to 37 °C

Fig.6. Plate API20E for the germs of the coliforme type



After the 24 hours incubation to 37 °C

Fig.7. Plate API20E for the germs of the Salmonella type

Although the biochemical tests constitute a classical approach for the identification of certain species and under species thanks to this test, it is possible to know the characteristics of the metabolisms of the isolated bacteria. During the identification, several medium are imply; Mannitol mobility, citrate of Simmons, test LDC (lysin décarboxylase), production of Indol, medium of Voges-Proskawer (VP) and ONPG. The biochemical tests were the object of our investigation for cinqechantillons. The whole of the got results is deferred in table n°3.

2.3.1 Results of identification: Stocks, of coliformes isolated starting from raw milk from cow highlighted 3 species which are klebsiella, proteus mirabilis and Escherichia coli, the other stocks of studied lactic bacteria are cram positive and negative. The biochemical identification of 5 stocks of lactic bacteria supports 2 groups: bacteria different from the lactobacillus kind; lacidophilus.

Table 3. Results of the biochemical characters of the stocks isolated from purified medium SS on Mac Conkey starting from the 5 attentive samples by two raw milk breedings

Test Souche	ONPG	Glu	Lac	H2S	Gaz	Mob	Man	Cit	Nit	LDC	ODC	ADH	IND	TDA	VP
1	+	+	-	+	+	+	+	-	+	+	+	+	-	-	+
2	-	+	+	-	+	+	+	-	+	-	-	+	+	+	+
3	+	-	-	-	+	+	-	-	+	+	-	+	+	-	-
4	-	-	+	+	+	+	-	-	-	-	+	-	-	+	+
5	+	+	-	-	+	+	+	-	+	+	-	+	+	-	-
6	-	-	-	-	+	+	-	-	+	-	+	-	-	+	
7	-	-	-	+	+	+	-	-	-	-	-	-	+	+	
8	+	-	+	-	+	+	-	-	+	+	+	+	-	-	-
9	-	+	-	-	+	+	+	-	-	+	-	+	+	+	+
10	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+

(+): Positive test; (-): Negative test.

Lime: glucose; Lake: lactose; Mob: mobility; Man: mannitol; Cit: citrate; Nit: nitrate; IND: indol; TDA: tryptophan désaminase; VP: Voges Pro

3. EXPERIMENTAL

3.1 products of Safilait

The Safilait dairy currently knows a development of diversified an enough range.

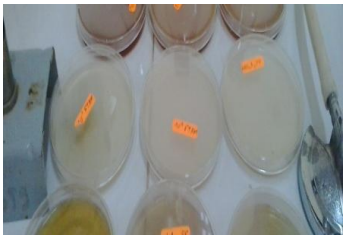
3.2 microbiological Analyses

Fig.8. Repared decimal dilutions

3.2.1 The research of the total aerobic micro-organisms (FTAM)

The micro-organisms develop in a nutritive medium agar PCA. [7]

A: Sowing in Mass agar PCA



B: Solidification on the straw mattress

C: after incubation

After the 72 hours incubation to 37 °C

Fig.9. Enumeration of the FTAM

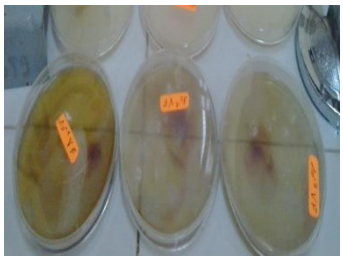
Reading of the results

Limp containers more than 300 colonies and month of 30 colonies [7],

3.2.2. The research of the clostridies

The micro-organisms develop in an agar medium grazes liver then one added a bulb of Alin of iron and 12.5 G of sulphite. Dilutions 10^{-1},10^{-2} and 10^{-3} are subjected initially to a heating with 80°c during 10 minutes then with an immediate cooling under the water of tap, with an

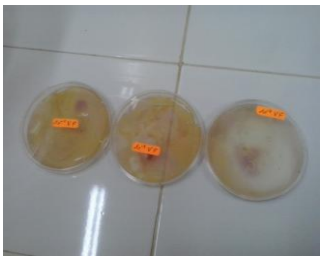
aim of eliminating the vegetative forms and from sporulés guards only the forms.



A: Sowing masses some Agar grazes liver

B: Incubation in the earthenware jar of anaérobiose

After the 24 hours incubation to 37 °c



C: After incubation

Fig.10. Enumeration of the clostridies

Reading of the results

It is absolutely necessary to locate any black colony having pushed in mass and one of a diameter higher than 0.5 mm.

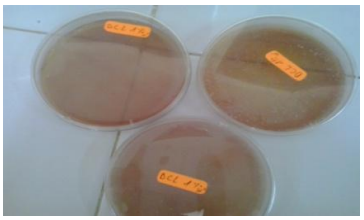
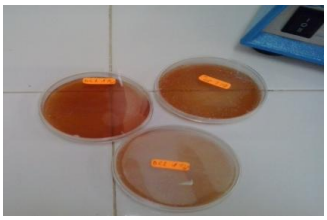
Table 4. Development of the micro-organisms for three dilutions

Dilution	**10^{-1}**	**10^{-2}**	**10^{-3}**
24h	0	0	0
48h	0	0	0
72h	0	0	0

3.2.3 The research of coliforme total and the coliformes

3.2.3.1 Total coliformes

For each dilution 1ml is sown in the mass of approximately 15 ml of agar DCL (désoxycholat) limps about it of Petri [7].

A: deposited agar DCL in limps B: solidification on the straw mattress C: after incubation

After the 24 hours incubation to 37 °C

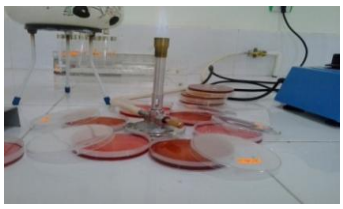
Fig.11. Enumeration of the total coliformes

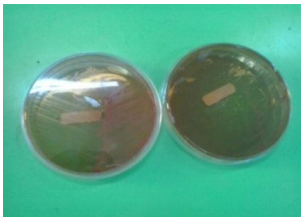
Table 5. Forms of the colonies according to the micro-organisms for coliformes total.

Colonies	**Micro-organisms**
Red in the shape of lenses with strip of precipitate	Lactose- E. coli
Stakes with pink center and halation of precipitate	Lactose-Entérobactérie Crepsilla and other
Colourless	Lactose-salmonellas, shigila Carrying and pseudomonas

3.2.3.2 Fecal coliformes

For the fecal coliformes by the same method of sowing on Makcanky agar,





A: deposited agar in limps B: solidification on the straw mattress C: after incubation

After the 24 hours incubation to 44 °C

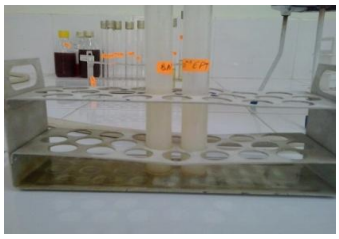
Fig. 12. Enumeration of the fecal coliformes

Reading of the results

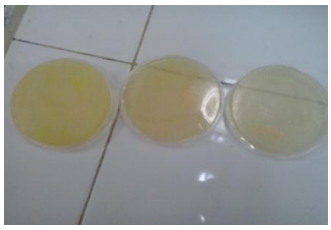
They are in the form of the large red colonies with turbid colony.

3.2.4 The search for Staphylococcus aureus

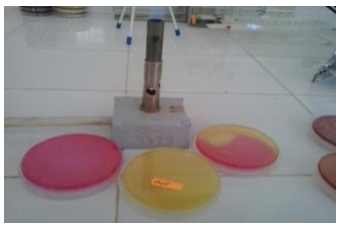
An insulation is carried out by sowing out of rake 0.1 ml on Baird-Parker agar,



A: Enrichment on the EPT



B: Sowing out of rake



C: After incubation

After the incubation of 24 during 48 hours with 37 °C

Fig.13. Search for staphylococcus aureus

Reading of the results

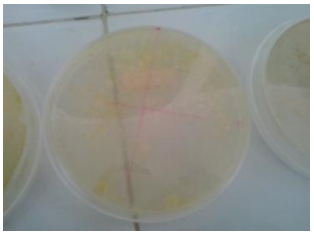
The staphylococcus aureus cultivates easily on solid medium, it forms colonies convex, luisantes and more or less pigmented in yellow.

3.2.5 The Research of the Salmonellas

To sow in three dials the solid medium of insulation: agar désoxycholate [7].

A: Sowing in three dials

B: solidification SS



C: Afterwards incubation

After the incubation from 24 to 48 hours with 37 °C

Fig.14. Research of the salmonellas

Table 6. Forms of the colonies according to the micro- organisms for salmonella

Colonies	Micro-organisms
Colourless and transparency	Shigila and more the share of the salmonellas
Rose with red	E. coli
Larger than E. coli pink with viscous opaque cream white	Enterobactery aerogenes
Transparency with center black	Enterobactery aerogenes

Reading of the results

Les salmonellas appear colourless and transparent of small**.**

4. CONCLUSION

Five raw milk samples of mixture were the object of our investigations microbiologiquesportant on 6 floresA through this research, we evaluated the degree of contamination of lamatière first, the cow's milk intended for the manufacturing of cheese with paste molletype Camembert. The microbiological analyses of the five samples of milk studied montrentl' complete lack of the germs searched except for FTAM (Total Germs Aerobic Mésophile, with a weak presence not exceeding the threshold of acceptability. Concerning the search for salmonellas and the staphilococca our results revealed the absence of this germ in all our samples. As regards analyses microbiology, the sontencourageants results, nevertheless vigilance and the rigour throughout the preparation remain dislocated at end of always the consumer a product of the first quality ensures. These results indicate that the samples of the raw milk of cow intended for the manufacturing of cheese with paste molletype Camembert cheese are of very good qualities from microbiological point of view. Consequently, we recommend to the company to increase the fréquencede these analyses and to apply the system of prevention, by (***Method HACCP***) in laiterieSafilait (at least once by quarter for equipment and once all 6 moispour the equipment of production). We plan to continue this work by the study of molecular modeling by theoretical methods of calculating

in order to optimize bacteriological lesquality of raw milks of several stock breeder.

5. ACKNOWLEDGEMENTS

Authors are thankful to LASNABIO laboratory for providing support for this work

6. REFERENCES

[1] Pougheon S. (2001). Contribution à l'étude des variations de la composition du lait et ses conséquences en technologie laitière. Thèse doctorat d'état en médecine vétérinaire, université Paul Sabatier de Toulouse, France.

[2] Goursaud J, (1985). Composition et propriétés physico-chimiques. Dans Laits et produits laitiers vache, brebis, chèvre. Tome 1 : Les laits de la mamelle à la laitière. Luquet F.M. Edition Tec et Doc Lavoisier, Paris.

[3] Vierling E, Aliments et boissons filières et produits. 3^{éme} édition Biosciences et techniques.Paris. 2008, 15-16

[4] Agabriel C, Coulon J.B, Brunschwig G, Sibra C, et Nafidi C. Relations entre la qualité du lait livré et les caractéristiques des exploitations. INRA Prod. Anim., 8 (4), 1995, 251-258

[5] Debry G. (2001). Lait, nutrition et santé. Edition Tec et Doc Lavoisier, Paris.

[6] FAO. (1995). Le lait et les produits laitiers dans la nutrition humaine. Collection FAO Alimentation et nutrition n°28. ISBN 92-5-20534-6

[7] Guiraud J.P, et Rosec J.P. Pratique des normes en microbiologie alimentaire. Edition AFNOR. 2004, 95, ISBN: 9782124452118

How to cite this article:
Mesli F, Lazouni Hamadi A. Biochemical and microbiological identification of cow's milk for the manufacture of soft cheese. J. Fundam. Appl. Sci., 2019, *11(3), 1228-1243.*