الجمهورية الجزائرية الديمقراطية الشعبية
Democratic and People's Republic of Algeria
وزارة التعليم العالي والبحث العلمي
Ministry of Higher Education and Scientific Research
جامعة 8 ماي 1945 قالمة
University 8 May 1945 Guelma
Faculty of Nature and Life Sciences, Earth and Universe Sciences

## Memory in view of obtaining the Master's Degree

**Field: Science of Nature and Life.**
**Chain: biological science.**
**Speciality/Option: applied microbiology.**
**Department: Ecology and environmental engineering.**

## Theme:

# Evaluation of The microbiological and physico-chemical quality of food product according to the shelf life (case of Harissa).

**Presented by:**

- **HADDAD INES**
- **MEHALAINE DOUNIA**

**In front of the committee member:**

| | | |
|---|---|---|
| **The superviser: MALEK. I** | **M.C.B** | **University 8 May 1945 Guelma** |
| **The president : HADDIDI. I** | **M.C.B** | **University 8 May 1945 Guelma** |
| **The examiner : TALEB. Y** | **M.C.B** | **University 8 May 1945 Guelma** |

**2023-2024**

# Thanksgiving

First of all, we give thanks to the Almighty for granting us the strength, perseverance and blessings necessary to carry out this modest work.

We would like to express our deep gratitude to Ms. **Malek Insaf**, our thesis director, for her wise supervision, her judicious advice and her unfailing support throughout this research. Her relevant observations and recommendations have allowed us to structure our reflection and deepen our analyses. We are grateful to her for the time she has devoted to us, for her patience, her trust, her encouragement and the attention she has paid to the smooth running of our work.

Our sincere thanks also go to the members of the jury, in this case Ms. **Haddidi Imen**, President, and Ms. **Taleb Yasmine**, Examiner, for agreeing to evaluate this thesis. We are very grateful to them for their valuable contributions and the fruitful exchanges we have had. Their wise comments have enriched our work.

A big thank you to our dear parents for their unconditional love, their constant encouragement, their moral and financial support during all these years of study. This success is also yours.

Finally, we thank all the people who, from near or far, have contributed to the realization of this modest work.

# Dedication

I thank Almighty Allah for my success in this hard work and diligence and for having patience and perseverance to make my dreams come true.

I dedicate this humble work to the dearest people in the world, my parents **Yasmina** and **Lahcene**, and I know that my success is their greatest gift.

To my Mother for her love, patience, support and staying by my side in the most difficult moments that I have experienced.

To my father for his love, his patience, especially the sacrifices he made for me, and his words of encouragement that pushed me to perseverance and success.

To My sister **Imen** and her husband **Bilal**, who have never left my side. I will always appreciate their efforts and what they have done for me.

To my dear brother **Aymen** who always helped me and supported me .

To my dear uncle **Alaoua** and my dear aunt **Nadjet** .

To my uncle's dear wife **Yamina**, and my sweet cousins **Houda**, **Hasna** and **Fairouze**.

To the whole **Haddad** and **Hamadi** family.

To **Dounia**, my sister, my friend, and my companion, for our beautiful friendship that was not only in the academic field. I would like to thank you for being by my side and supporting me, and I hope that this friendship will not end, God Almighty willing.

To all my loved ones whom I did not mention.

Last but not least I want to thank me for believing in me, I want to thank me for doing all this hard work, for having no days off, I want to thank me for never quitting, I want to thank me for just being me all the times.

*Haddad Ines*

# Dedication

I thank Almighty Allah for my success in this hard work and diligence and for having patience and perseverance to make my dreams come true.

I dedicate this humble work to the dearest people in the world, my parents **Samira** and **Abde Arahmane** know that my success is their greatest gift.

To my Mother for her love, patience, support and beeing by my side in the most difficult moments that I have experienced.

To my father for his love, his patience, especially the sacrifices he made for me and the words of encouragement that pushed me to perseverance and success

To My sweet little Sisters Lina and **Rouya**.

To my Dear Brother **Hamza**.

To my dear grandmother, **Aziza**.

To my uncles and my aunts and especially my uncle **Saber**.

To the whole **Mehalaine** and **Boukahil** family.

To **Ines**, my sister, my friend, and my companion, for our beautiful friendship that was not only in the academic field. I would like to thank you for being by my side and supporting me, and I hope that this friendship will not end, God Almighty willing.

To all my loved ones whom I did not mention.

Last but not least I want to thank me for believing in me, I want to thank me for doing all this hard work, for having no days off, I want to thank me for never quitting, I want to thank me for just being me all the times.

*Mehalaine Dounia*

## List of abbreviations and acronyms

| | Symbols |
|---|---|
| % | Percentage. |
| ° C | Degree Celsius. |
| > | Superior. |
| < | Lower. |
| (+) | Positive character. |
| (- ) | Negative character. |
| = | Equal. |
| | A |
| ASR<br>AFNOR | Sulfito-reducing anaerobes.<br>French Association for standardization. |
| | C |
| CT<br>CF | Total Coliforms.<br>Fecal coliforms. |
| | D |
| D/C | Double concentration. |
| | E |
| EC<br>E.coli | Electrical conductivity.<br>Escherichia coli. |
| | F |
| FTAM | Total Aerobic Mesophilic Flora. |
| | G |
| Galerie<br>g | Analytical profile index.<br>Gram. |
| | H |
| H | Hour. |
| | I |
| ISO | International organization of standardists. |
| | J |
| JORA | Official Journal of the Algerian Republic. |
| | M |
| ML | Milliliter. |
| | O |
| OGA | Oxytetracycline-Glucose-Agar. |
| | P |

| | |
|---|---|
| **PCA**<br>**PH** | **Plate Count Agar.**<br>**Potential hydrogen.** |
| | **S** |
| **SF** | **Fecal Streptococci.** |
| **SS** | **Salmonella-Shigella.** |
| **S/C** | **Simple concentration.** |
| | **T** |
| **T** | **Temperature.** |
| | **U** |
| **UFC** | **Colony-forming UNIT.** |
| | **V** |
| **VF**<br>**VRBL** | **Liver Meat Agar.**<br>**lactose agar mixed with purple crystal and neutral red.** |

# Figures List

**Figure 1**: The harissa . ..... 4
**Figure 2**: Red pepper . ..... 4
**Figure 3**: Harissa Purea . ..... 4
**Figure 4**: Longitudinal section of a chilli. ..... 5
**Figure 5**: Chemical Structure of Capsaicin . ..... 7
**Figure 6**: Chili puree ..... 12
**Figure 7**: Harissa ingredients ..... 13
**Figure 8**: Olive oil . ..... 14
**Figure 9**: Organizational chart of the ABIDI company. ..... 20
**Figure 10:** Products of the company "ABIDI". ..... 21
**Figure 11**: Weighing of the pepper at the entrance . ..... 22
**Figure 12**: Reception of hot pepper. ..... 22
**Figure 13**: Washing the chili pepper . ..... 23
**Figure 14**: Control by manual sorting ..... 23
**Figure 15**: The preheater ..... 24
**Figure 16**: The boilers . ..... 25
**Figure 17**: Dilution of the concentrated pulp ..... 25
**Figure 18**: Filling and crimping of the boxes ..... 26
**Figure 19**: Sterilization and cooling tunnel of the harissa . ..... 27
**Figure 20**: Packaging and storage of boxes . ..... 27
**Figure 21**: Benchtop PH meter before and after storage ..... 32
**Figure 22:** Measure dry residue "Brix" of the Harissa ..... 33
**Figure 23**: The samples of harissa ..... 38
**Figure 24**: Harissa ingredients ..... 39
**Figure 25**: Preparation of the stock solution ..... 40
**Figure 26**: Filtration of samples ..... 40
**Figure 27:** University microbilogy laboratory ..... 41
**Figure 28**: Ph-meter used in the laboratory ..... 42
**Figure 29**: Determination of pH by pH meter ..... 43
**Figure 30**: Determination of conductivity by conductivity meter ..... 44
**Figure 31**: Determination of BRIX by refractometer ..... 46

**Figure 32**: Determination of acidity ..... 47
**Figure 33**: Determination of ash contents ..... 49
**Figure 34**: Preparation of the stock solution ..... 50
**Figure 35**: Dilution technique ..... 51
**Figure 36:** preparation of the culture medium..... 52
**Figure 37**: Search for total mesophilic aerobic germs ..... 53
**Figure 38:** Search for yeasts and molds . ..... 55
**Figure 39**: Research protocol and enumeration of coliforms . ..... 56
**Figure 40**: Search and enumeration of fecal Streptococci ..... 57
**Figure 41**: Research and enumeration of anaerobic sulfito-reducing bacteria (ASR) ..... 58
**Figure 42**: The search for staphylococci ..... 61
**Figure 43**: The Search for Pseudomonas..... 62
**Figure 44**: The Search for Shigelles ..... 63
**Figure 45**: Oxidase Search. ..... 64
**Figure 46**: Catalase search..... 65
**Figure 47**: Preparation of the API 20TH gallery . ..... 66
**Figure 48**: API 20E positive vs negative ..... 67
**Figure 49**: API 20NE positive vs negative ..... 69
**Figure 50** : API Staph positive vs negative . ..... 70
**Figure 51**: Histogram of the pH of the analyzed samples as a function of time. ..... 73
**Figure 52**: Histogram of the Conductivity of the analyzed samples as a function of time. .... 74
**Figure 53**: Histogram of the Brix of the analyzed samples as a function of time. ..... 76
**Figure 54**: Histogram of the titratable acidity of the analyzed samples as a function of time.78
**Figure 55**: Histogram of the ash content of the analyzed samples as a function of time. ..... 80
**Figure 56**: Graphical presentation of the results of the FTAM enumeration. ..... 82
**Figure 57**: Representing the search for FTAM on PCA ..... 83
**Figure 58**: Graphical presentation of the results of the yeasts and molds. ..... 84
**Figure 59**: Representing the search for the yeasts and molds on OGA ..... 85
**Figure 60**: Graphical presentation of the results of the Total coliforms. ..... 86
**Figure 61**: Representing the search for the Total coliforms on VRBL . ..... 88
**Figure 62**: Representing the search for the Staphylococci on chapman..... 91
**Figure 63**: Representing the results of the API galleries ..... 93

# Tables List

**Table 1**: Descreption of the harissa . ..... 5
**Table 2**: The international Cronquist classification for chili pepper. ..... 6
**Table 3**: Morphological differences between the main species of peppers ..... 6
**Table 4**: Nutritional value of harissa per 100g ..... 7
**Table 5**: Fatty acid composition of an olive oil. ..... 15
**Table 6**: Technical data sheet of the company. ..... 19
**Table 7**: The sampling dates. ..... 38
**Table 8**: Amounts of olive oil added. ..... 38
**Table 9**: Nutritional values of the harissa. ..... 39
**Table 10:** The reagents to be added to API 20E. ..... 66
**Table 11:** The reagents to be added to the API 20 NE. ..... 68
**Table 12**: The reagents to be added to the Staph API. ..... 69
**Table 13**: The pH values of harissa studied. ..... 73
**Table 14**: The Conductivity values of harissa studied. ..... 74
**Table 15**: The Brix values of harissa studied. ..... 76
**Table 16:** The titratable acidity values of harissa studied. ..... 77
**Table 17**: The ash content values of harissa studied. ..... 79
**Table 18:** Numbers of the counted germs of total mesophilic aerobic germs. ..... 82
**Table 19:** Numbers of the counted germs of yeasts and molds. ..... 83
**Table 20**: Numbers of the counted germs of Total coliforms. ..... 86
**Table 21**: Numbers of the counted germs of fecal coliforms. ..... 88
**Table 22**: Numbers of the counted germs of Fecal streptococci. ..... 89
**Table 23**: The enumeration of the sulphito-reducing Clostridium ..... 89
**Table 24:** The enumeration of Salmonella. ..... 90
**Table 25:** The enumeration of Staphylococci. ..... 90
**Table 26**: Result of the biochemical tests (API gallery, catalse test, oxidase test). ..... 93

## Table of Contents

**Thanksgiving**

**Dedication**

**Abbreviations List**

**Figures List**

**Tables List**

**General Introduction** ..... 1

**Theoretical part**

**Chapter I: Generality on harissa and olive oil**

**I.1.Origin and history of harissa** ..... 3

**I.2.Definition** ..... 4

**I.3.Classification** ..... 5

**I.4.Chemical composition of harissa** ..... 6

**I.5.Dietary and nutritional importance of the harissa** ..... 7

**I.6.Agents for improving the quality of canned food** ..... 9

**I.7.How to choose and store harissa** ..... 12

**I.8.Definition of olive oil** ..... 13

**I.9.Classification of olive oil** ..... 14

**I.10.Composition of olive oil** ..... 15

**I.11.The role of olive oil in food preservation** ..... 16

**Chapter II: Processes of production, transformation and conservation of harissa**

**II.1.Presentation of the transformation unit** ..... 19

**II.1.1.Origin and geographical distribution of the plant** ..... 19

**II.1.2.Technical sheet of the company** ..... 19

**II.1.3.Organization chart of the ABIDI company** ..... 20

**II.1.4.The activities of the ABIDI canned food company** ..... 20

**II.2. Concentrated harissa production process** ..... 21

**II.2.1.Preliminary operations** ..... 21

**II.2.2. Transformation** ..... 23

**II.2.3 Harissa manufacturing diagram** ..... 28
**II.2.4 Quality control** ..... 29
**II.3. Physico-chemical analysis of canned harissa** ..... 30
**II.3.1. External reading of the packaging and labeling of the cans** ..... 30
**II.3.2. Weight control** ..... 31
**II. 3.3. Hydrogen potential (ph)** ..... 31
**II.3.4. Temperature control** ..... 32
**II.3.5. Determination of the (BRIX)** ..... 32
**II. 3.6. Determination of viscosity** ..... 33
**II.3.7. Determination of acidity** ..... 34

## Experimental part

## Chapter III: Material and methods

**III.1.Materials used during the present study** ..... 37
**III.2.Choice of harissa variety** ..... 37
**III.2.1. The nutritional composition of Harissa** ..... 39
**III.3.Sampling** ..... 39
**III.3.1.Sample Preparation** ..... 40
**III.4.Methodes of analysis** ..... 41
**III. 5. Physico-chemical analyses** ..... 42
**III.5. 1. Determination of the hydrogen potential (pH)** ..... 42
**III.5.2.Determination of conductivity** ..... 43
**III.5.3.Determination of BRIX degree** ..... 45
**III.5.4.Determination of titratable acidity** ..... 46
**III.5.5.Determination of the ash content** ..... 47
**III.6.Microbiological analyses of harissa concentrate** ..... 49
**III.6.1.sample preparation** ..... 50
**III.6.2.Preparation of decimal dilutions** ..... 50
**III.7. Microbiological analysis methods** ..... 51
**III.7.1. Search and enumeration of total mesophilic aerobic germs** ..... 52
**III.7.2.Research and enumeration of yeasts and molds** ..... 54
**III.7.3.Research and enumeration of total and fecal coliforms** ..... 55
**III.7.4. Search for fecal streptococci** ..... 56
**III. 7. 5. Research and enumeration of anaerobic sulfito-reducing bacteria (ASR)** ..... 58
**III.7.6. Search for pathogenic germs** ..... 59

**III.8. Biochemical identifications of germs** ..... 63
**III.8.1. Search for oxidase and catalase** ..... 63
**III.8.2. API Galleries** ..... 65

## Chapter IV : Results and Discussion

**IV.1.Results of physico-chemical parameters** ..... 72
**IV.1.1.Determination of pH** ..... 72
**IV.1.2.Determination of conductivity** ..... 74
**IV.1.3.Determination of BRIX degree** ..... 75
**IV.1.4.Determination of the titratable acidity** ..... 77
**IV.1.5.Determination of ash content** ..... 79
**IV.2. Microbiologic analyzes** ..... 81
**IV.2.1. Effect of olive oil and time on the development of microorganisms** ..... 81
**IV.2.2. Microbiological analysis results** ..... 81
**Conclusion** ..... 94
**Bibliographic references**
**Abstract**
**Résumé**
**ملخص**
**Annex**

# General Introduction

# General Introduction

Plant-based agri-food products play an essential role in the human diet due to their richness in nutrients crucial to meet nutritional needs. As highlighted by the Food and Agriculture Organization of the United Nations **(FAO, 2020)**.

Processed food products, in particular canned food, are particularly sensitive to microbial development and to alterations in their physico-chemical properties. This is why a rigorous control of their quality is essential in order to ensure their stability and their safety for the consumer. As the World Health Organization **(WHO, 2021)**.

Among the vegetable culinary specialties of the Mediterranean region, the harissa holds a place of choice. This spicy paste emblematic of Maghreb cuisines, mainly Tunisian, Moroccan and Algerian, is a must for North African gastronomy **(Daoussi et *al*., 2022)**. Composed essentially of dried hot peppers, garlic, coriander, caraway and olive oil, harissa gives a spicy and spicy flavor to the dishes to which it is added. More than just a condiment, it embodies the ancestral Arab-Berber culinary identity and occupies a major cultural role in these countries **(Dhouioui et *al*., 2022)**.

Since ancient times, man has been looking for methods to preserve perishable vegetables, thus guaranteeing their availability in times of shortage and limiting losses due to improper storage. Some families and industrialists' resort to preserving in olive oil, benefiting from its protective properties. According to the definition of the Codex Alimentarius, "olive oil consists of a mixture of glycerides, most of which is formed by unsaturated fatty acids, obtained only by mechanical processes or other physical processes, under thermal conditions that do not lead to the alteration of the oil" **(Codex Stan 33-1981, 2021)**.

Extra virgin olive oil, due to its rich composition in antioxidant compounds such as polyphenols, tocopherols and carotenoids, has remarkable properties for preserving the quality of food. As **(Squillari et *al*., 2023)** point out in their review published in 2023 in the journal Antioxidants, the use of olive oil as an ingredient or frying/cooking medium can contribute to slowing down the lipid oxidation and degradation processes, while preserving the desired organoleptic and nutritional qualities of food products. Its antioxidant and antimicrobial potential therefore make it an ingredient of choice to improve the shelf life and stability of food.

The incorporation of olive oil in food preparations such as preserves, marinades or other culinary preparations not only prolongs their shelf life, but also enriches their nutritional

profile. Due to its composition rich in monounsaturated fatty acids and natural antioxidant compounds, olive oil gives better stability to foods, slowing down degradation processes **(Dabbou et *al*., 2021)**. Its judicious use in processing processes thus contributes to preserving the organoleptic qualities and the nutritional value of the products for a longer time. At the same time, they benefit from the recognized health benefits of olive oil, making it an ingredient of choice for a healthy and sustainable diet **(Martín-Peláez et *al*., 2021)**.

A regular evaluation of the microbiological and physico-chemical parameters throughout the storage period is crucial to guarantee the quality and safety of the product. As **(Carrillo et *al*., 2022)** demonstrated in 2022, carrying out analyzes at different stages of the life cycle makes it possible to quickly detect any microbiological contamination or deterioration of the product. Regular checks during storage make it possible to ensure that the production processes meet hygiene standards and good manufacturing practices.

The main objective of this study is to evaluate the evolution of the microbiological and physico-chemical quality of the harissa as a function of its shelf life. It aims to analyze the changes that occur in the characteristics of the product, both microbiologically and physicochemically, over the storage time.

The content of this work is divided into two main parts :

The first part of this manuscript is devoted to a bibliographic synthesis comprising 2 chapters:

- The first chapter concerns a generality on harissa and olive oil.
- The second chapter presents the production processes, transformation and conservation of harissa.

The second part of this work consists of an experimental study divided into two chapters:

- The third chapter will be dedicated to the detailed description of the equipment and methodologies used to carry out the various analyses and controls carried out as part of this study. The chapter present in depth the experimental techniques and protocols implemented within the university's laboratories to evaluate the microbiological, physicochemical parameters and other aspects of the quality of the product studied.
- At the end, the last chapter mentions the various results obtained during our study in the form of tables and graphs, with a discussion and a conclusion.

# Theoretical part

# Chapter I

# Generality on harissa and olive oil

## I.1.Origin and history of harissa

Harissa is a chili pepper puree that originates from Tunisia. Bell peppers are dried in the sun and ground along with spices such as cumin, coriander and caraway, not forgetting sun-dried tomatoes. However, harissa can also be prepared using fresh bell peppers or boiled and mixed bell peppers. Harissa is usually sold in cans, but it is also available in tubes**[1]**.

Native to South and Central America, the chili pepper is the fruit of the Capsicum which is declined through a huge family. In addition to its use in cooking, chili is also used for its antiseptic properties. This is also why it is also widespread in all tropical cuisines. It is also a very rich source of vitamin A and C, magnesium and iron **[2]**.

Chili peppers originate from Central and South America. All wild species (approximately 25 species) can be found in this area. Cultural forms were already domesticated in prehistoric times **(Fondio, 2009)**.

The cultivation of chili peppers is very old. It is thought to originate from Brazil. It was one of the first plants cultivated in South America (Mexico) 7000 years ago. Chili peppers are used as spices or vegetables due to their multiple properties (medicinal, culinary, etc.). It was only at the end of the 15th century, after the voyages of Christopher Columbus, that they were introduced to Europe. Discovered by the Spaniards in Santo Domingo, pepper quickly became the "poor man's spice." In fact, imported spices were very expensive in his 17th and his 18th centuries and were outwardly symbols of wealth. This pepper replaced the very expensive "Indian pepper" **(Khineche E, 2022)**.

Spaniards traveling from America brought chili peppers to Tunisia, providing Tunisians with the basis for harissa spice mixtures. From there, harissa quickly spread to all countries in the Maghreb region of North Africa. This mixture is also used in breakfast foods **[3]**.

**Figure 1**: The harissa **[4]**.

**Figure 2**: Red pepper **[5]**.

## I.2.Definition

The Harissa comes from the Arabic word "harasa" which means "to crush". It is indeed a condiment originating from Tunisia, obtained by grinding red peppers, very popular in Middle Eastern cuisine **[6]**.

Harissa is a red pepper puree originating from Tunisia and has become a true emblem of Maghreb cuisine. Traditionally, to obtain the harissa, the red pepper is dried in the sun and then finely ground. Depending on the recipes and the regions, garlic, fresh or dried tomatoes and various spices and herbs are added to the red pepper puree thus obtained: salt, coriander, caraway, cumin, mint, etc. In France, harissa is found in the majority of supermarkets packaged in tubes or canned **[6]**.

**Figure 3**: Harissa Purea **[7]**.

**Table 1**: Descreption of the harissa **(Boussaid F et *al*., 2019)**.

| Product name | Harissa |
|---|---|
| Origine of the product | fresh chillies |
| Ingredients | Fresh chillies +salt+spices+crushed garlic |
| Packaging | put in a metal box |
| Storage condition | Keep cool after between 3-4°C |
| Shelf time | Between the date of manufacteur and the date of expiry |
| Intended use | To be served in the cooked state |
| Place where product will be sold | Retail selles and large surfaces |
| Presentation | 1/6 (135g) 1⁄2 (380g) 4/4 (760g) |

## I.3.Classification

The chili pepper is the fruit of the variable species of the genus Capsicum which belongs to the Solanaceae family of which this family also includes tomato, eggplant and potato The fruits of the chili pepper are considered vegetables, but botanically speaking, they are berries, in fact, they are generally classified according to the characteristics of fruits (pungent taste, color, shape of fruits, etc.) **( Khineche E, 2022)**.

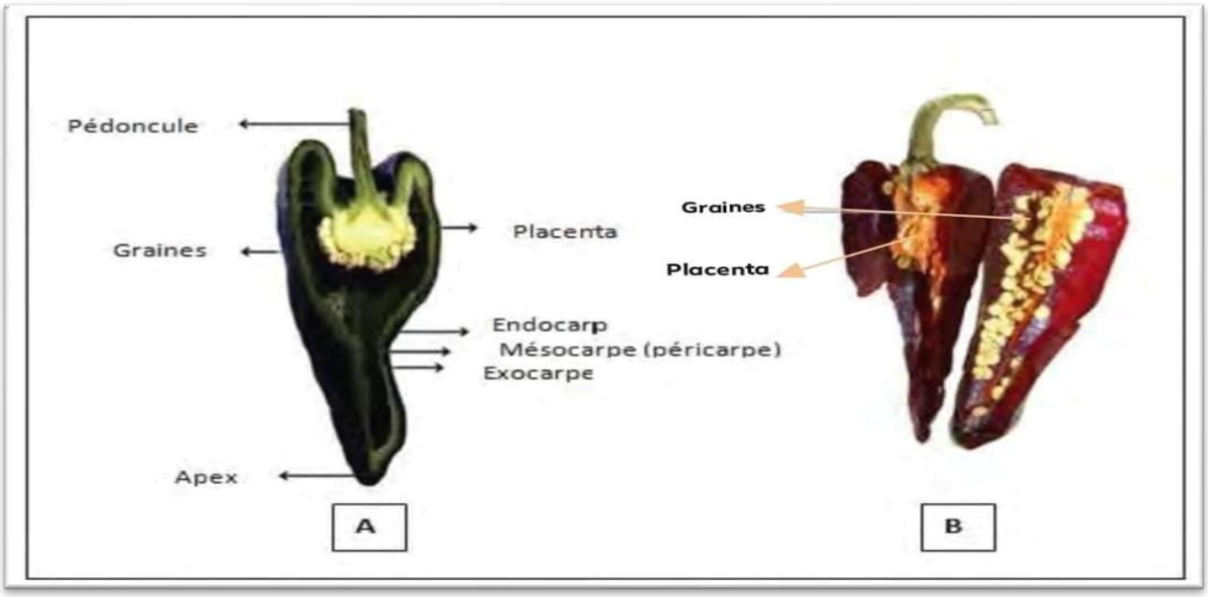



**Figure 4**: Longitudinal section of a chilli.

**A**: fresh green chilli; **B**: dried red chilli **(Tellez Perez, 2013)**.

**Table 2**: The international Cronquist classification for chili pepper **(Azrou and Amaouz, 2020)**.

| | |
|---|---|
| **Reign** | Plantae |
| **Under the reign** | Tracheobionta |
| **Subdivision** | Spermatophyta |
| **Division** | Magniolophyta |
| **Class** | Magniolopsida |
| **Subclass** | Asteridae |
| **Order** | Solanales |
| **Family** | Solanaceae |
| **Sex** | Capsicum |
| **Species** | Capsicumannuum |

The genus Capsicum includes 25 wild and 5 domesticated species (C. annuum L., C. frutescens L., C. chinense Jacq, C. baccatum Jacq, and C. pubescens L.), which include more than 200 varieties, but there are few varieties that have been studied in detail **(Pugliese et *al*., 2012)**. Capsicum annuum is the most widespread and cultivated species in temperate and subtropical countries **(Kothari et *al*., 2010)**.

**Table 3**: Morphological differences between the main species of peppers **(Krishna De, 2004)**.

| Species | Color of flowers | Number of knots/flower | Seed Color | Calcium Constriction |
|---|---|---|---|---|
| ***C. annuum*** | White | 1 | yellowish | **absent** |
| ***C. frutescents*** | Green | 2-5 | yellowish | **absent** |
| ***C. chinense*** | White/greenish | 2-5 | yellowish | **absent** |
| ***C. baccatum*** | White with yellow spots | 1-2 | yellowish | **absent** |
| ***C. pubescens*** | Purple | 1-2 | black | **absent** |

## I.4.Chemical composition of harissa

The chemical composition of harissa typically includes red chili peppers, garlic, salt, cumin, coriander, and sometimes mint.

The chemical composition of chili pepper is mainly characterized by the presence of capsaicin. The capsaicin molecule with the formula $C_{18}H_{27}NO_3$ is undoubtedly the element that allowed the chili pepper to spread all over the world. Indeed, the chili owes its spiciness to the capsaicinoids which are present in this plant to eliminate and scare away its natural

predators. The spiciness of the chili pepper varies depending on the amount of capsaicinoid **[8]**.

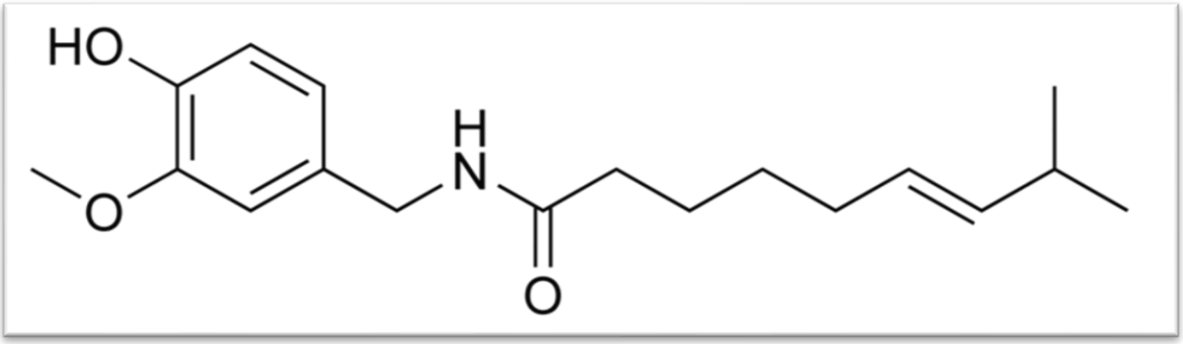



**Figure 5**: Chemical Structure of Capsaicin **[9]**.

**Ingredients % :**

- Red pepper 86%.
- Garlic extract: 4% in pods.
- Caraway seeds: 2%.
- Coriander: 4%.
- Salt (NaCl): Max 4% of the product weight **[10]**.

## I.5.Dietary and nutritional importance of the harissa

### ➢ The calories and nutritional information of the harissa

Harissa is pretty good for your health. Composed of capsaicin (alkaloid) which gives it its pungent taste, it is an antioxidant that protects the body from certain diseases, such as diabetes, certain cancers and cardiovascular diseases **[11]**.

This pepper has a lot of nutritional qualities because it is low in calories, rich in fiber, vitamins B6, C, E and K. If harissa promotes digestion and burns fats and sugars by its combustible action, you should not abuse it too much or not have a fragile stomach **[11]**.

**Table 4**: Nutritional value of harissa per 100g **[11]**.

| **Nutritional value of harissa per 100g** | |
|---|---|
| **Proteins** | 2.7g |
| **Carbohydrates** | 7.1g |
| **Lipids** | 2.9g |
| **Calories** | 76kcal |

- **The health and nutrition benefits of harissa**

Harissa is the ideal condiment to give taste to a dish without having to add fats or industrial sauces. Rich in fiber, it is low in calories, naturally contains very few carbohydrates and is devoid of fats. Also, it allows you to take advantage of the advantages of red chili rich in vitamins Band C. The capsaicin contained in chili and, by extension, in harissa is a powerful antioxidant which makes it an ally of choice as part of a varied and balanced diet.

The consumption of harissa stimulates the salivary glands and digestive enzymes, thus facilitating digestion. Finally, the warming action of chili pepper stimulates the metabolism and the burning of fats and sugar. However, if you have a sensitive stomach, be careful not to overdo it **[12]**.

- **Health benefits of harissa**

Consumed regularly, harissa is famous for allowing the body to burn more fat. Indeed, the harissa :

* Stimulates the production of saliva from the beginning of digestion,
* Allows a greater release of digestive enzymes,
* Accelerates the digestion process,
* Stimulates the conversion of fats into energy,
* Protects the body from infectious diseases such as colds and viruses, especially during the winter **[13]**.

Its consumption promotes digestion, strengthens the immune system and reduces inflammation. But as in everything, beware of excesses. In addition, capsaicin, one of its components, is an antioxidant, good for health. Harissa is low in calories, rich in fiber and vitamins **[14]**.

- Harissa is the ideal condiment to give taste to a dish without having to add fats or industrial sauces. Rich in fiber, it is low in calories, naturally contains very few carbohydrates and is devoid of fats. Also, it allows you to take advantage of the advantages of red chili rich in vitamins B and C **[15]**.

The capsaicin contained in chili and, by extension, in harissa is a powerful antioxidant which makes it an ally of choice as part of a varied and balanced diet. The consumption of harissa stimulates the salivary glands and digestive enzymes, thus facilitating digestion. Finally, the warming action of chili pepper stimulates the metabolism and the burning of fats and sugar. However, if you have a sensitive stomach, be careful not to abuse it **[15]**.

## I.6.Agents for improving the quality of canned food

Preservatives are chemical substances added to food to achieve the following effects :

- Prevent their degradation;
- Improve their appearance;
- Reserve their nutritional qualities.

Some preservatives come from natural sources such as salt, sugar, vinegar and lemon juice. Others are manufactured or artificial.

There are many types of preservatives. Here are some examples :

- Benzoic acid.
- Calcium sorbate.
- Isoascorbic acid.
- Potassium nitrate.
- Sodium benzoate.

Antioxidants can also be used as preservatives in order to slow down the degradation process of a product. Here are some examples:

- Ascorbyl palmitate.
- Butyl hydroxyanisole (BHA).
- Butyl hydroxytoluene (BHT) **[16]**.

Food preservation methods have developed following the evolution of man and his lifestyle. There are three methods for preserving food in order to preserve its taste properties and edibility. Among these methods, several techniques exist **[17]**:

### ❖ Heat preservation techniques

The first technique is that of heat preservation, which can first go through pasteurization. It is a question of subjecting the food to a temperature between 85°C and 100°C, to then cool it abruptly, with the aim of destroying pathogenic microorganisms and alteration. This first method of preservation has the advantage of preserving the flavor of foods which, moreover, have a shelf life and must be kept cool.

The second technique is that of sterilization which consists in the passage of food at a temperature above 100°C to destroy any microbial form. Subsequently, the foods that have been subjected to this sterilization can be stored at room temperature.

The third technique is that of ultra-high temperature (UHT) treatment. The food, which can be stored for a long time, at room temperature, is brought to more than 135°C for a very short time and is then quickly cooled and packaged aseptically.

Appertization is also a conservation method that combines two techniques. That of packaging in a can, and a technical treatment, most generally sterilization. The food that will be canned will thus be able to be stored for several years at room temperature. The particularity is that once the minimum durability date has been exceeded, the food remains consumable, it will simply have lost its taste and nutritional quality **[17]**.

- **Cold storage techniques**

Cold storage is a known technique for preserving food. The cold makes it possible to slow down or stop the activity of the cells of the food, to prevent the development of microorganisms and to prolong its lifespan. However, as long as the microorganisms are not destroyed, once the food is at room temperature, they can resume their activity. In this case, the consumption of these foods can pose a risk to the health of individuals.

Refrigeration is one of these cold storage techniques. It consists in lowering the temperature of the food between 0°C and +4°C, to slow down cellular metabolisms, the cells of animal and plant tissues remain alive. It makes it possible to extend the shelf life of food.

Freezing is another technique that allows the temperature of the food to be lowered in such a way as to turn the water it contains into a solid state and to slow down or even stop the enzymatic and microbial activity **[17]**.

- **Additives**

An additive is any substance that is not normally consumed as a foodstuff, nor normally used as a characteristic ingredient of a foodstuff, whether or not it has a nutritional value, and whose intentional addition to a foodstuff for a technological purpose at any stage of the manufacture, processing, preparation, packaging, transport or storage of said foodstuff entails, or may, in all likelihood, lead (directly or indirectly) to its incorporation or that of its

derivatives in this food or otherwise affect its characteristics **(CODEX STAN 192-1995, 2015)**.

They allow in particular :

* To help conservation by preventing the presence and development of undesirable microorganisms (for example: molds or bacteria responsible of food poisoning) they are called preservatives.

* To avoid or reduce the oxidation phenomena that cause, among other things rancidity of fats (alteration of fats exposed to air, light and heat) or browning of cut fruits and vegetables.They are called anti oxygen.

* To improve the presentation or the hold, they are called texturing agents (emulsifiers, stabilizers, thickeners, gelling agents).

* To give back to food, to strengthen or to impart a coloration, they are called dyes.

* To strengthen their taste (flavor enhancers). The sweeteners thus provide a sweet taste **(Apab, December 2011)**.

- **Thickeners and gelling agents**

The thickening and gelling agents are chemical in nature linear or branched polymer chains with hydrophilic groups which enter into physical interaction with the water present in the product. It makes it possible to obtain the desired consistency of food, improve and maintain the structure of products. With the performance of other technological functions :

stabilizers and humectant. The gelling and thickening agents are carbohydrates (polysaccharides) of vegetable origin, vegetable hydro colloids **(Doublier, 2009).**

- **Dyes**

The main function of food dyes is to impart, strengthen or preserve the color of food and beverages. They make it possible to provide a desired coloration, to accentuate an existing shade or to compensate for a loss of color caused by various factors such as prolonged exposure to intense light, high temperatures or thermal variations. When added to foodstuffs,

these additives have the role of uniformizing the shade and preventing premature discoloration.

The use of these dyes essentially meets aesthetic criteria, with the aim of making food more visually attractive and appetizing for consumers. Their objective is to optimize the visual aspect of food products in order to make them more enticing, thus stimulating the appetite and the desire for consumption.

### I.7.How to choose and store harissa

Food preservation is a set of processing methods that make it possible to preserve the taste and nutritional properties, texture and color characteristics foodstuffs. It also aims to preserve their edibility by delaying the growth of bacteria in order to avoid possible food poisoning. Finally, she allows to delay the oxidation of fats which causes rancidity **[18]**.

You have the choice between jarred, canned or tube harissa, it's up to you to choose what is most convenient for your use. If you opt for the "homemade", then choose dried chillies **[19]**.

**Figure 6**: Chili puree **[20]**.

Before opening, the harissa can be stored for up to 3 years in a dry place protected from light. After opening, you can store your harissa for up to 1 month in the refrigerator in its original packaging or in an airtight container in order to avoid the proliferation of microorganisms in the product **[19]**.

Harissa is a chili-based preparation that can be stored for quite a long time if stored properly. To preserve the harissa, it is important to keep it away from light, heat and moisture.

It is also recommended to store it in an airtight container, such as a glass jar with a metal lid **[20]**.

In general, a well-preserved harissa can be stored for up to 1 year after its production date, but it is preferable to consume it within 6 months to make the most of its flavors **[20]**.

You can pack your homemade harissa in a glass jar. Stored in the refrigerator, it can last for a month. However, be sure to always use a clean spoon for service **[20]**.

**Figure 7**: Harissa ingredients **[20]**.

## I.8.Definition of olive oil

Olive oil is the oil coming only from the fruit of the olive tree (Olea europaea L.) to the exclusion of oils obtained by solvent or by re-esterification processes and of any mixture with oils of other nature **[21]**.

Olive oil is the main source of fat. It is a virgin oil extracted from the fruit of the olive tree (Olea europaea) only by mechanical processes or other physical processes **[22]**, consumable in its raw form without prior treatment.

**Figure 8**: Olive oil **[23]**.

Olive oil is a vegetable oil obtained by pressing olives. This oil is rich in Omega-9 and has many virtues for our health **[24]**.

- **Olive oil identity card**
- ✓ Common name of vegetable oil: Olive ;
- ✓ Producer tree: Olive tree ;
- ✓ Botanical name: Olea europaea var. europea. Under the species "Olea europea", we can essentially find 3 subspecies: var. europea (studied on this sheet) which is its cultivated form, then var. silvestris Brot. and var. africana that are a wild form of it ;
- ✓ Botanical family: Oleaceae ;
- ✓ Origin (country where the vegetable oil is grown): Spain, Greece, Italy, South of France, Tunisia ;
- ✓ Part of the plant extracted: Fruit (pulp of olives) ;
- ✓ Oxidative potential: Not very sensitive **[24]**.

## I.9.Classification of olive oil

The olive tree belongs to the Oleaceae family with 30 different species according to the classification of **(Lousert and Brousse, 1978)**.

According to **(Ghedira, 2008)**,The botanical classification of the species (Olea europea L) is the only species whose fruits are edible is as follows :

- Kingdom: Plantae.

- Branching: Magnoliophyta.
- Subfamily: Magnoliophytina.
- Class: Magnoliopsida (Dicotyledonous).
- Subclass: Dialypetals.
- Order: Lamiales.
- Family: Oleaceae.
- Genus : Olea.
- Species: Olea europaea.
- Subspecies: O.europea subsp.europaea var. sylvestris O.europea subsp.europaea var. europaea.

## I.10.Composition of olive oil

Olive oil is a complex mixture of different chemical compounds and like all vegetable oils, olive oil consists of a saponifiable fraction called main or major and an unsaponifiable fraction called secondary or minor **(Giuffre, 2013)**.

❖ **The saponifiable fractions**

**1. Fatty acids**

Fatty acids are the basic components of most lipids, **(Giuffre, 2013)**, often found bound to glycerol to form triglycerides or free after the hydrolysis of the latter. In vegetable oils, the fatty acids mainly consist of 16 and 18 carbon atoms.

**Table 5**: Fatty acid composition of an olive oil **(Veillet, 2010)**.

| fatty acids | raw formula | Olivier et coll (%) | codex alimentarius (%) |
|---|---|---|---|
| **Myristic acid** | C14 :0 | Tr | <0.1 |
| **Palmitic acid** | C16 :0 | 7.5-15.6 | 7.5-20 |
| **Palmitoleic acid** | C16 :Ln7 | 0.3-1.9 | 0.3-3.5 |
| **Margaric acid** | C17 :0 | <0.3 | <0.5 |
| **Margaroleic acid** | C17 :Ln-8 | <0.5 | <0.6 |
| **Stearic acid** | C18 :0 | 1.4-3.4 | 0.5-5 |
| **Oleic acid** | C18 :Ln-9 | 60.9-82.1 | 55-83 |
| **Vaccinic acid** | C18 :Ln-7 | 0.7-3.6 | - |
| **Linoleic acid** | C18 :2n-6 | 4.5-16.6 | 3.5-21 |

| | | | |
|---|---|---|---|
| **Linolenic acid** | C18 :3n-3 | 0.4-1.2 | <1.5 |
| **Arachidonic acid** | C20:0 | 0.3-0.5 | <1.8 |
| **Gadoleic acid** | C20 :Ln-9 | 0.2-0.5 | - |
| **Behenic acid** | C22 :0 | <0.2 | <0.2 |
| **Lignoceric acid** | C24 :0 | <0.1 | <1 |

**2. Triglycerides (TG)**

They form between 98 to 98.5% of the mass of the olive oil, are triesters resulting from the combination of three molecules of AG by their carboxyl function with the alcoholic functions of glycerol **(Rouas et *al.*, 2016)**.

❖ **Composition of the unsaponifiable fraction**

The unsaponifiable corresponds to the sum of the components of a fatty substance which, after saponification, they are responsible for the taste and the unique fragrance of this product, It represents 0.4 to 0.8% of olive oil, These ingredients can be separated into phenolic compounds, tocopherols, pigments, aromatic compounds, terpene hydrocarbons and sterols **(Metlef, 2021)**.

## I.11.The role of olive oil in food preservation

With freezers and refrigerators, easy access to ready-to-use food, we have forgotten the preservation techniques of our grandparents: jars, lacto-fermentation, jams, candied fruits, salting, etc. At a time when packaged foods are being pointed out because of, on the one hand, ultra-processing and additives that are bad for your health and, on the other hand, their ecological impact, it is useful to relearn these old techniques that have proven their worth. Here we will talk about the use of oil for preserving **[25]**.

The oil prevents mold from growing in food by surrounding it with a protective film. It is also a natural antioxidant that stops the oxidation of food, in particular by preventing air contact with food. Once completely immersed in oil, vegetables and other foods keep for several years, It is recommended to use olive oil because it is both good for health and an excellent natural preservative thanks to its antioxidants **[25]** , Olive oil has antibacterial properties (olive oil contains many nutrients that can inhibit or kill harmful bacteria) **[26]**.

These benefits have been linked to its well-balanced fatty acid composition, where oleic acid C18 is the main component and to the presence of minor compounds, such as vitamins and natural antioxidants **(Benabid, 2009)**.

For the preservation of Products in oil, Extra Virgin Olive Oil is considered the best product ever. The use of extra virgin olive oil for the conservation of food products seems to be one of the best choices, especially for all the properties that make it particularly suitable for conservation **[27]**.

# Chapter II

# Processes of production, transformation and conservation of harissa

## II.1.Presentation of the transformation unit

### II.1.1.Origin and geographical distribution of the plant

The ZIMBA factory is a family business of the ABIDI group, created in 2000 and located in the industrial zone of the municipality of Belkheir Wilaya of Guelma, near the national road 80 leading to Sedrata; it has about 400 employees in various fields **(Foughali, 2021)**.

The unit has been manufacturing tomato concentrate based on fresh tomatoes since August 2010. Its production capacity is 450 t/day of fresh tomato. Tomato is supplied from 140 farmers. Its capacity has been increased with the realization of a second chain of 600t / day from August 2016. The products, carefully developed by this cannery are simple and double tomato concentrate, jam and chillies (Harissa) **(Bennacer, 2018)**.

### II.1.2.Technical sheet of the company

**Table 6**: Technical data sheet of the company.

| Company name | The ABIDI preserves |
|---|---|
| **Company headquarters** | **Industrial zone Guelma** |
| **Legal Form** | **SARL Company** |
| **Date of activity** | **2002** |
| **Plant area** | **4100 m2 and 700m2 for production ; 2900 for storage** |
| **Address** | **SADRATA Road BP 26 BELKHEIR - wilaya of GUELMA.** |
| **Tel** | **+213.30.72.22.22** |
| **E-mail** | **ABIDI GUELMA@Yahoo.fr** |
| **Production capacity** | **Tomato paste: 450 Ton/Day**<br>**Jam: 150 Ton/Day**<br>**Harissa: 350 Ton/Day** |
| **Turnover** | **6930698 74** |
| **Tax identifier** | **001024038282176** |

### II.1.3.Organization chart of the ABIDI company

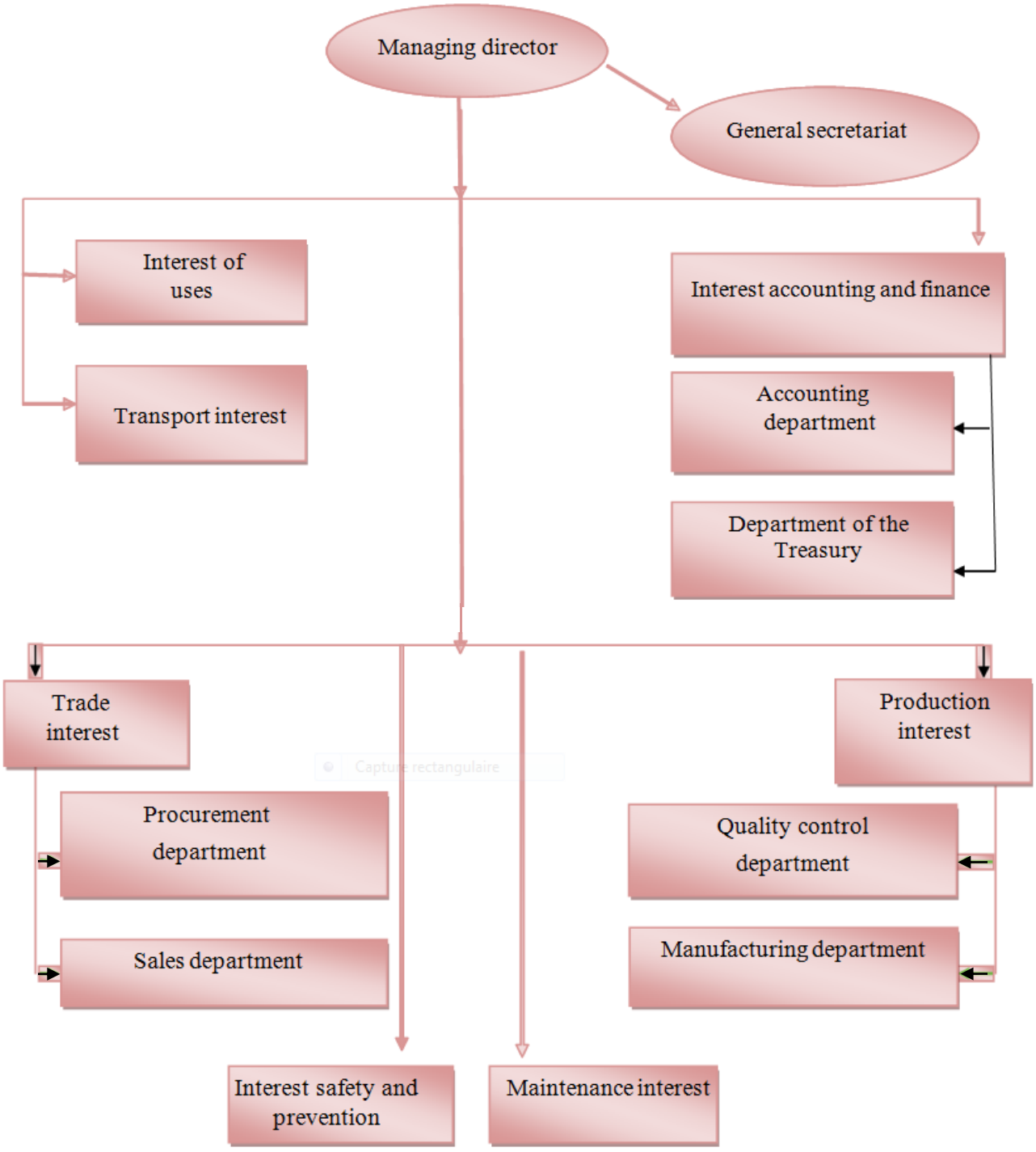



**Figure 9**: Organizational chart of the ABIDI company.

### II.1.4.The activities of the ABIDI canned food company

The company's activity includes the manufacture of several products, namely :

- For jam preserves: strawberries, peach.

- Wash the canned tomatoes.
- Add the canned chilli.
- For the production of flour.


(a)**[29]**.


(b)**[29]**.


(c)**[29]**.


(b)**[28]**.

**Figure 10:** Products of the company "ABIDI".

**a**: Jam (strawberry and peach...), **b**: tomato and harissa, **c**: Flour.

This company plays important roles in the regional economy :

At the regional level: The company participates in the sale of agricultural products because it consumes a significant amount of tomatoes, fruits and grains from the wilaya of Guelma and other wilayas **[30]**.

## II.2. Concentrated harissa production process

### II.2.1.Preliminary operations

1. **Weighing**

Before entering the processing chain, it is necessary to know the number of the incoming (input). A rocker is used for weighing the starter peppers.

**Figure 11**: Weighing of the pepper at the entrance **(Bordjiba.k et *al*., 2023)**.

**2. Reception**

The peppers are received by the workers, the crates are unloaded and samples are taken to determine their quality. After cleaning, juice is extracted from the samples, to determine the percentage of Brix of the pepper received.

**Figure 12**: Reception of hot pepper **(Bordjiba.k et *al*., 2023)**.

**3. Storage of raw materials**

It is necessary to keep the chilli fresh for a certain time, necessary for its ripening before adding it to the processing chain. Just leave the chilli in a well-ventilated place.

**4. Washing**

This procedure is necessary to remove the "soil", which may include residues of spraying, microorganisms, contaminants, moisture, rodents and eggs and drosophila larvae. The water used for this washing must be drinkable.

**Figure 13**: Washing the chili pepper **(Bordjiba.k et *al*., 2023)**.

5. **Sorting and trimming**

The washed peppers are carefully sorted, so that only the good ones are sent to the grinder and that defective and poor-quality peppers (such as soggy areas, wetlands and insect-damaged areas) be sent back.

**Figure 14**: Control by manual sorting **(Bordjiba.k et *al*., 2023)**.

### II.2.2. Transformation

1. **Grinding**

After rinsing, the peppers pass through a grinder at a temperature of the order of 70°C, in which the peppers are compressed between two rollers so as to cause the liquid to flow of the chambers of the fruit containing the seeds.

The separation of this liquid from the rest of the pepper is done by passage through a rotary screening. Finally, the seeds are separated from the liquid that coats by centrifugal effect. The peppers from the seed separator are fed to the grinder.

The crushed product is collected in tanks equipped with a discharge pump to the preheating step.

**2. Preheating**

The preheating operation makes it possible to ensure a pre-cooking of the pulp in order to facilitate the output of the juice in the refining unit, it also allows the reduction of the microbial load.

The preheating is carried out at a temperature of 80°C. And at a pressure of 1 bar in a horizontal tubular exchanger which consists of bundles in which the ground material circulates.

**Figure 15**: The preheater **(Bordjiba.k et *al*., 2023)**.

**3. Refining**

This operation consists in extracting the juice from the crushed (it is a separation liquid-solid carried out using a system of rasping blades (strainers) for 12 hours.

The material first is conveyed to the turbo extractor (strainer) in which a rotor provided with blades radial exerts a centrifugal action against a conical sieve allowing the separation of the skins, seeds and other impurities of the juice.

The juice seeps through the strainer (the intermediate product of the harissa product), then that the solid matter is eliminated (debris, seeds and pepper pulps).

**4. Concentration**

This process takes place after sieving, it reduces the amount of active water which prolongs the life of the harissa, concentrates it by partial evaporation and precipitates it to the evaporator. The two controlled variables are the temperature and the Brix.

**Figure 16**: The boilers **(Bordjiba.k et *al*., 2023)**.

When the primary ingredient (fresh chilli) is present in sufficient quantity throughout the along the campaign, the factory constitutes reserves of semi-finished product which will be recycled out of the countryside and sold. For this reason, the product is condensed to a Brix of 42% (triple concentrated), then it enters the sterilization and heat treatment process for the rid of all microorganisms, after which comes the stage of stuffing and storage in aseptic airless bags to keep the product as long as possible.

Outside the campaign period, the plant's experts transfer the semi-finished product to consumption by diluting it and improving its Brix content by adding water Treaty.

**Figure 17**: Dilution of the concentrated pulp **(Bordjiba.k et *al*., 2023)**.

Subsequently and after having obtained a minimum concentration of 14%, the spices (salt, coriander, caraway ...) previously sieved, crushed and refined to enhance the taste.

### 5. Pasteurization

Is a heat treatment process to inactivate the enzymes and kill the relatively heat-sensitive microorganisms that cause deterioration, with minimal changes in the properties of food.

The harissa concentrate passes into a pasteurizer where it undergoes a heat treatment at 92°C.

### 6. Filling and crimping

The pasteurized product is pumped to the metering crimping machine, this set of two synchronous machines, ensures the filling and then the crimping of the metal boxes at a well-defined speed that varies from one format to another.

The cans are turned over several times and cleaned with steam at 90°C.

Before to be filled in order to destroy the germs that may be present there. They then arrive at the station filling. The filling machine is a device with about 40 heads each, rotating at one speed adjusted according to the needs and whose maximum is 300 cans per minute, then the crimping which is done in a four-headed crimper and at a speed proportional to that of the filling; consists in closing the cans hermetically on the underside after the filling with harissa concentrate at 90°C.

At the exit of the crimper, a date written on on the same side the date of manufacture, expiration, the time of release of the product and the number of the batch to which the box belongs.

**Figure 18**: Filling and crimping of the boxes **(Bordjiba.k et *al*., 2023)**.

### 7. Sterilization and cooling

The cans are sterilized using a large rotary sterilizer which gives the continuous the box a heat treatment, this heat treatment makes it possible to preserve the product of all bacteria without altering its nutritional value.

Then they are done in a double tunnel portion. In the first part, sterilization takes place by direct injection on the cans of hot water. For this, the water is heated to 96°C in a tubular exchanger, then sent in the sterilizer by shower jets, on the boxes that line up on a wide conveyor belt at low speed. The hot water used is collected and recycled. In the second part of

the tunnel, cold water is injected onto the cans for cooling to 40°C. it is called a shock thermal.

It is used to avoid over-cooking, and to tell about the phenomena of internal corrosion of the boxes. The water is collected and sent to the refrigerant.

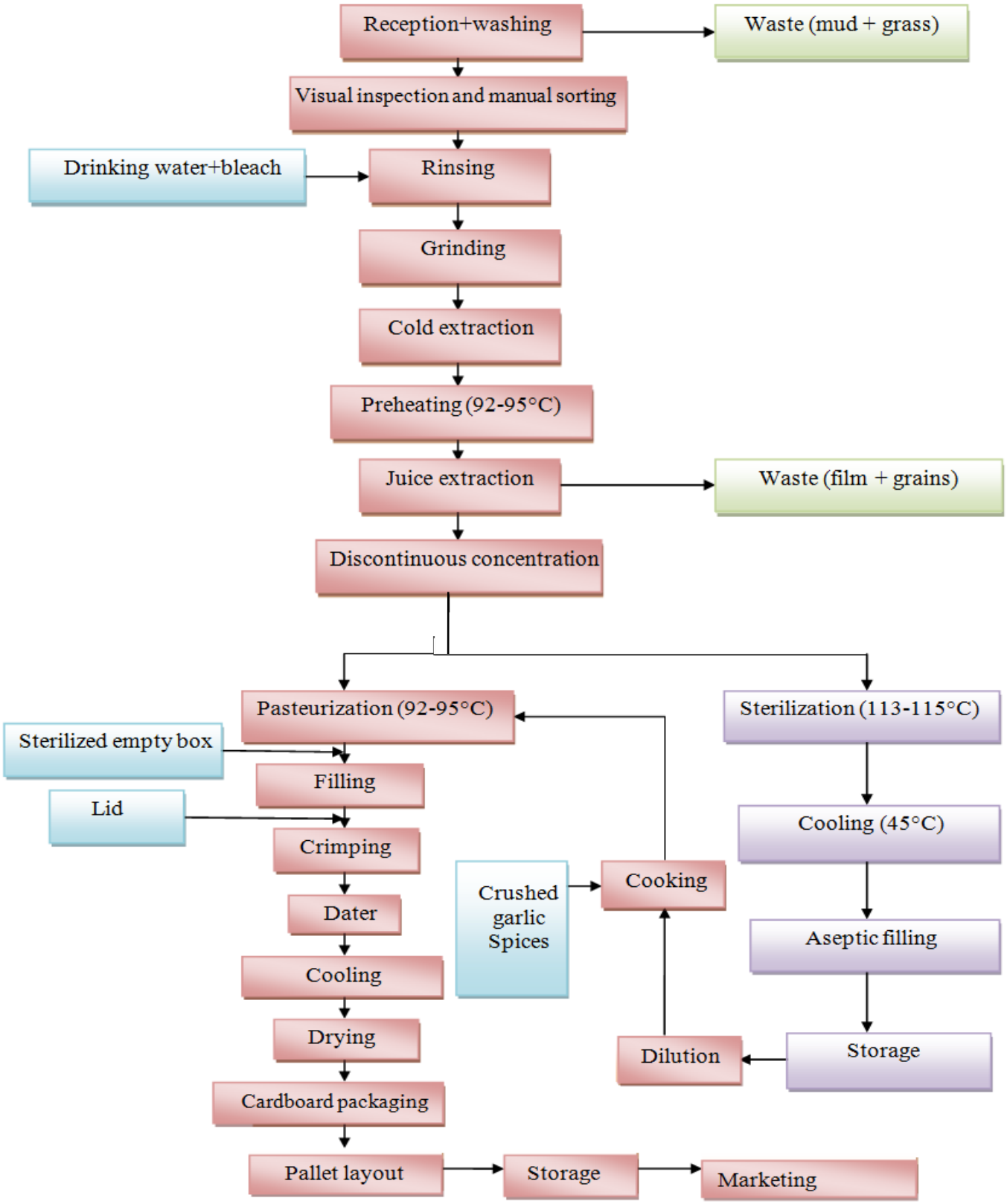
**Figure 19**: Sterilization and cooling tunnel of the harissa **(Bordjiba.k et *al*., 2023)**.

## 8. Packaging and storage

At the exit of the tunnel, a dryer injects hot air on the boxes. These last properties Dried, are packaged automatically in boxes of 12 for the boxes of 800g and 24 for that of 400g. These boxes are taped and stored in the sheds before to be delivered to the market at least 21 days after the date of manufacture; which allows to complete the analyses of the product in the laboratory.

**Figure 20**: Packaging and storage of boxes **(Bordjiba.k et *al*., 2023)**.

### II.2.3 Harissa manufacturing diagram



### II.2.4 Quality control

Because the products offered have a direct impact on public health, the agri-food industry is an area where quality control is essential.

In general, it will be important to ensure that the various transformations preserve the nutritional and biological properties of the harissa, to do this, the control will be done on several levels.

1. **Raw material control**

He already starts with the purchase of the raw material with a consequent choice. At control and quality laboratory, Brix and pH are measured in order to predict the behavior from the product to the transformation. This control is continued after washing by visual sorting.

It will consist essentially of eliminating unripe peppers, infected by molds or bacteria and if possible cut off the parts involved. This section will be the prerogative of maneuvers but under the responsibility of a permanent agent of the factory in order to bring possible corrections.

The quality of the concentrate depends on that of the chili pepper.

2. **Manufacturing control**

Compliance with the specifications of the experts and the equipment manufacturer is important here. The temperature, pressure, supply water quality and Brix levels will be all under surveillance. Accordingly, during the implementation, a control manual will be created to list all the procedures and checks to be carried out at each stage of the process.

3. **Control of the finished product**

It will focus on the physical, organic and chemical characteristics on the one hand and on the stability and quality of service on the other hand.

4. **Crimping control**

If the pasteurization is not carried out correctly, the harissa paste will escape from the container. This is already a standard check.

The same phenomenon (pressure difference between inside and outside) can also be observed during quenching. Otherwise, we can assume that the service is accurate. However, it is impossible to predict whether it will last long time.

**5. Stability control**

It is necessary to keep samples of each production in the laboratory and to examine them for a while. If the box is not opened, we can assume that the contents are intact. For the controls of the concentration characteristics, it is necessary to make sure of :

- The color that must be red,
- The texture and consistency,
- The flavor and aroma.

## II.3. Physico-chemical analysis of canned harissa

This present experimental study consists in following the measurement of the physicochemical parameters, and stability of the finished product at the factory level.

The laboratory is a room equipped with the necessary installations and devices for manipulations and the experiments carried out, intended to control the product during the various stages of the manufacturing, it must be a very active sector within the unit to avoid any questions about quality of the product. According to our internship at the ABIDI group company, the parameters they perform are the following :

### II.3.1. External reading of the packaging and labeling of the cans

A food can presents a certain number of information that will have to be checked before to carry out physicochemical or microbiological analyses. We will quote :

The mandatory information notices :

- ✓ Product name – Sales denomination – Brand.
- ✓ Composition – List of ingredients - Possible allergens present (quantitative).
- ✓ Net weight or net volume.
- ✓ Product category and minimum concentration (Dry extract or Brix).
- ✓ Date of manufacture.
- ✓ Expiration date (DLUO for certified canned goods of vegetable origin at pH<4.5).
- ✓ Batch number.
- ✓ Name of the production plant and postal contact details (address, telephone, fax email, website).
- ✓ Place of origin (country of origin if imported).
- ✓ The type of packaging used is also carefully noted according to its characteristics.

### II.3.2. Weight control

Weighing an object is measuring the mass of an object with a scale. Generally speaking :

- The tare: it is the "weight" of the packaging (or the container).
- The "net weight" is that of the product (we must say: "net mass").
- The "gross weight" is that of the packaged product (we must say: "gross mass").
- The operating procedure
- Then we weigh the box of the finished product (harissa).
- Then we take the empty box and weigh it.
- Then we do the subtraction of the two weights.

### II. 3.3. Hydrogen potential (ph)

It expresses whether the harissa is acidic or alkaline. It has no hygienic meaning, but it represents a very important notion for the determination of the aggressiveness of the harissa.

It defines in in addition to the belonging of the product to the different categories of canned food classified according to the pH = 4.5 or < 4.5 The pH of the harissa in double concentrate must be between 4.20 and 4.50. It must not in any case go beyond 4.60.

The determination of the pH of the hrissa derivatives is carried out electrometrically using a mechanical pH.

***The Operating procedure**

- All The harissa cans are first brought to room temperature (20-25°C).

- Then the pH measurement is carried out on the harissa puree by direct immersion of the probe in the harissa puree.

- A preliminary homogenization of the contents of the box is carried out using a spatula metallic.

- Then the pH meter is calibrated using buffer solutions.

- The temperature of the product is measured by means of a thermometer and the instrument is adjusted to this temperature. The pH is indicated directly by the device.


**Figure 21**: Benchtop PH meter before and after storage **(Personal photo 2024)**.

### II.3.4. Temperature control

For the finished product (box after cooling), the temperature is measured after opening the harissa box is filled on the surface and in the center of the box thanks to a thermometer. We are waiting for the the value displayed on the screen is stabilized.

### II.3.5. Determination of the (BRIX)

Brix is the main technological parameter in harissa concentrates. It represents the degree concentration of harissa. This parameter is subject to very strict regulations **(JORA, 1997).**

Brix is defined as being the sucrose concentration of an aqueous solution having the same refractive index as the analyzed product. A refractometer, equipped with a graduated scale indicating the refractive index.

***The operating procedure**

- To bring the test solution or the sample to a temperature close to that of measurement.

- Mix the sample well in order to make it homogeneous.

- To place about 10 grams of the product in the center of a square of canvas, gather the corners of the square .

- So as to enclose the test socket in a small part and press it gradually in order to exude liquid through the canvas.

- Drop a few drops on the measuring prism of the refractometer; fold down the prism lighting on the measuring prism by pressing it well against the latter, and proceed to the reading.

•To bring the line dividing the light and dark areas of the surface of the field of view to the intersection of the wires of the reticle and read the value of the refractive index or the percentage by mass of sucrose, depending on the device used.

• Then the result must be expressed as a percentage of soluble dry matter or Brix.

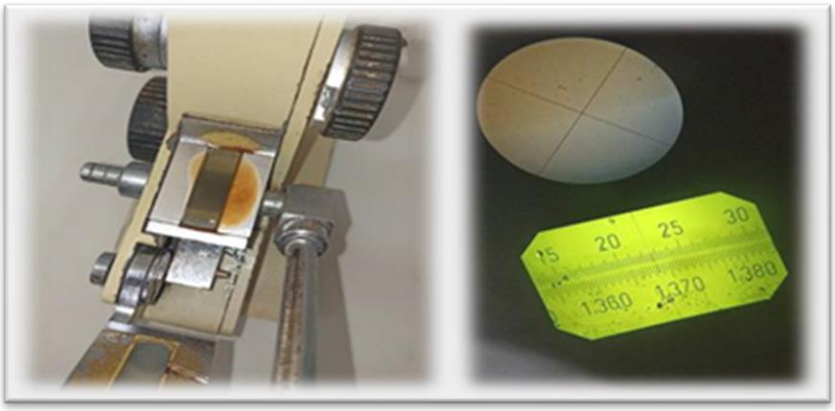



**Figure 22:** Measure dry residue "Brix" of the Harissa **(Boughida.M et *al*., 2023)**.

**II. 3.6. Determination of viscosity**

For all samples (samples during production and the finished product), the viscosity is determined using a Bostwick Consistometer, at an ambient temperature of 20°C. to 12.5% Brix.

The playback is performed after 30 seconds.

***Operating procedure**

• We locate the two screws on the back of the device until the bubble of the spirit level placed on the front of the device be centered. We close the compartment door.

• The sample to be evaluated is poured into the compartment and the surplus is evacuated using a Spatula.

• The product is released by pressing the lever arm downwards. The product is allowed to flow along from the slope for 30 seconds.

• We examine the distance traveled by the product along the slope during these 30 seconds. The slope is provided with graduations indicating the distance traveled in centimeters. We record this value as being the consistency of this product.The Bostwick Consistometer is cleaned and the device is dried properly before reuse.

- The Bostwick Consistometer is cleaned and the device is dried properly before reuse.

### II.3.7. Determination of acidity

This test allows the determination of the amount of acids present in the products to be analyzed (acidity natural + developed acidity), thus reflecting the acidic compounds of a solution.

The acidity we provides information on the condition of the product: on the severity of microbiological alterations. The purpose of this analysis is to approximately measure the total acid content of the product natural by a basic dosage with sodium hydroxide (NaOH) at 0.1molar.

***The operating procedure**

- 10g of product are weighed in a glass beaker, then 100ml of distilled water are added.
- The mixture is stirred well and it is transferred into a 200ml flask, and adjusted to 200ml with the distilled water is stirred again and then filtered.
- We take 50ml of the filtrate, we put it in a 1liter beaker.
- It is diluted with 300ml of distilled water, and two to three drops of phenolphthalein are added.
- It is titrated with sodium hydroxide (NaOH) until the persistent pink tint changes.
- The stirrer is started, and soda is added dropwise using a burette.
- When there is a color change, we add a drop and stop, We note the volume poured of soda v.

# Experimental part

# Chapter III

# Material and methods

### III.1.Materials used during the present study

These are the materials used in all microbiological and physico-chemical analysis laboratories of a food product:

- **Biological material**

The raw material is concentrated harissa and olive oil.

- **Laboratory equipment**
  - **Reagents**

    distilled water, paraffin oil, sodium sulphate, iron alum, oxygenated water.

  - **Equipment**
    - Glassware: test tubes, erlenmeyer flask, 250ml bottle, beakers, pasteur pipette, volumetric flask, graduated burette , spatula, test tube, funnels.
    - Disposable Plastic Transfer : Pipettes, micropipette, spatula, petri dishes. bunsen burner, inoculation loop, whatman paper.

- **Apparatus**

- Sterilization and incubation equipment 30°C, 37°C and 44°C, autoclave.
- The precision balance for weighing.
- The magnetic stirrer for homogenization.
- Water baths for the regeneration of environments.
- PH meter, vortex, incubator, muffle furnace.
- Refrigerator, refractometer, An electrical conductivity meter.

### III.2.Choice of harissa variety

Harissa is one of the most popular food stuffs on the Algerian market and the most consumed, so we selected a sample of X Harissa , for its study in order to assess the physical, chemical and microbiological quality of the Harissa and depending on the availability of means, we conducted three types of analyzes for four cans of the same type of Harissa selected: physical and chemical analyzes (determination of pH, Electrical conductivity, microbiological analyzes (search for the total average aerobic flora, E.coli).


**Figure 23**: The samples of harissa **(Personal photo 2024)**.

The purpose of this control is to assess the microbiological, physico-chemical quality of canned food. It allows the laboratory to decide on the possible presence or absence of microorganisms in the harissa concentrate, How and what is the impact range of olive oil affects on harissa and its role in maintaining its quality.

Cans of harissa are opened at the same time on 19/02/2024 (T=0) at room temperature. The four cans are analyzed on site (physico-chemical and microbiological analyzes), then olive oil is added in different quantities and stored in the refrigerator at a temperature of 4°C (recommended temperature) in order to identify changes in their sensory and health qualities after the seventh day of opening (T1 = 7 days), the fifteenth day (T2= 15 days), and finally a month later (T3=30 days) and finally the experiment was carried out according to the conditions of laboratory opening.

**Table 7**: The sampling dates.

| The time (days) | The sampling date |
|---|---|
| **T0** | 19/02/2024 |
| **T1** | 28/02/2024 |
| **T2** | 04/03/2024 |
| **T3** | 17/03/2024 |

**Table 8**: Amounts of olive oil added.

| The samples | Quantity of olive oil |
|---|---|
| **Witness (T)** | 0ml |
| **Sample 1 (E1)** | 10ml |
| **Sample 2 (E2)** | 15ml |
| **Sample 3 (E3)** | 20ml |

### III.2.1. The nutritional composition of Harissa

The Harrisa is of plant origin and consists of red pepper, Garlic extract ,Caraway seeds, Coriander ,Salt .

The harissa manufacturer in 20/11/2023 at 14.32 h and the consumption deadline and 19/11/2026.


**Figure 24**: Harissa ingredients **(Personal photo 2024)**.

**Table 9**: Nutritional values of the harissa.

| Nutritional information | Tenure in 100 g of harissa |
|---|---|
| **Energy** | 6.5g |
| **Lipids** | 5.8g |
| **Carbohydrates** | 4.6g |
| **Of which protein sugar** | 1.5g |
| **Sodium** | 0.5g |
| **Salt** | 1.25g |

### III.3.Sampling

We chose four cans of Harissa from the same production line, where they differ from each other in the amount of added olive oil and then we put the samples in the refrigerator at 4°C to extend 30 days.

We used these samples during a month of laboratory practice to determine the physical chemical parameters and perform a number of microbial analyses.

### III.3.1.Sample Preparation

**Preparation of the stock solution**

The stock solution is prepared under aseptic conditions from a sample of 25g of product (concentrated harissa) for 4 cans (witness, sample 1, sample 2 and sample 3) and homogenized with 225ml of distilled water. The stock solution is considered $10^{-1}$.


**Figure 25**: Preparation of the stock solution **(Personal photo 2024)**.

Before the process, the operation of filtration of harissa stock solutions (figure 26) made in sterile instruments.

**Definition of filtration**

filtration consists in passing a liquid through filters or porous substances that retain solid elements and microbes suspended in this liquid this is the sterilization method for all substances and culture media that are altered by heat **(N.Guezlane_tebibel et *al* ., 2008)**.

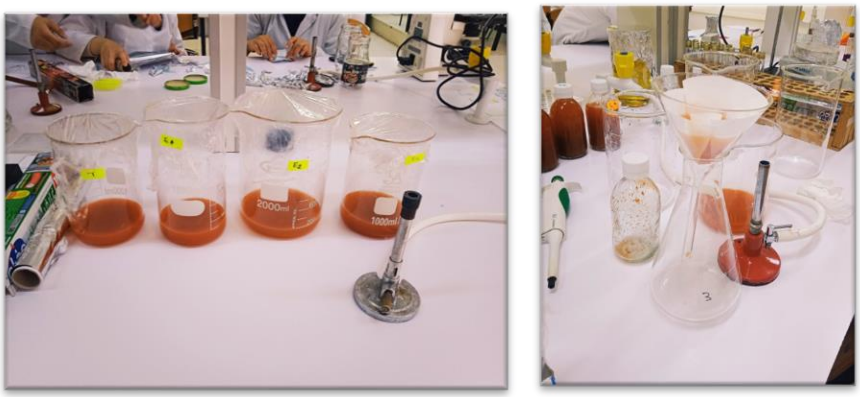



**Figure 26**: Filtration of samples **(Personal photo 2024)**.

## III.4.Methodes of analysis

Our study was carried out at the microbiology laboratory level (3,5,6) for 1 month, at the faculty of nature and life sciences and earth and universe sciences (SNVSTU) of the University of Guelma May 8,1945.

During this practice, we followed the determination of the physicochemical values and made a microbiological count.

**Figure 27:** University microbiology laboratory **(Personal photo 2024)**.

| Physicochemical analysis | Microbial analysis |
|---|---|
| - PH/temperature<br>- Conductivity<br>- Degree of BRIX<br>- Titratable acidity<br>- ash rate | - Total mesophilic aerobic germs<br>- Yeasts and molds<br>- Total and fecal coliforms<br>- Fecal *streptococci*<br>- Anaerobic sulphito-reducing bacteria (ASR)<br>- *Staphylococci*<br>- *Pseudomonas*<br>- *Shigella*<br>- *Vibrio cholera* |

## III. 5. Physico-chemical analyses

### III.5. 1. Determination of the hydrogen potential (pH)

In our study, we used a pH meter with automatic temperature display.

- **Principle**

The pH measurement is carried out by potentiometry **(Normes NF V76-122, 1994)**.

The hydrogen potential makes it possible to evaluate the concentration of the hydrogen ion in a solution **[31]**.

- **Equipment**

A pH meter is a device for measuring the pH of a solution **[32]**.

- First bring the samples to room temperature.
- To measure the pH of the product :
- Homogenize the contents of the can using a spatula.
- Immerse the pH meter probe directly into the box.
- Perform a calibration of the pH meter with pH buffer solutions 7 and 4.
- Measure the temperature of the product with a thermometer: Adjust the pH measuring instrument to this temperature.

The combined electrode is immersed in the product without dilution **(K. Horri et *al*., 2015)**.


**Figure 28**: Ph-meter used in the laboratory **(Personal photo 2024)**.

After calibration, use a pH meter to measure the pH by dipping the probe of the device

directly into the harissa sample to be analyzed. Read once the displayed value is stable.

❖ **Operating procedure**

- Press the ON/OFF button,
- We calibrate the pH meter,
- Rinse the pH meter probe with distilled water and dry it gently,
- Introduce it into the product to be analyzed (harissa),
- We are waiting for the value to stabilize,
- We note the value,
- Rinse the probe with distilled water, dry and turn off.


**Figure 29**: Determination of pH by pH meter **(Personal photo 2024)**.

**NB:** The experiment is repeated four times (4 samples).

### III.5.2.Determination of conductivity

Conductivity is a measure of the ability of an aqueous solution to conduct electric current. This property is related to the presence and concentration of electrically charged ions in the water. The more ions the solution contains, the better its ability to transmit current between two immersed electrodes**[33]**.

➢ **Equipment**

A conductivity meter is an instrument for quantifying the electrical conductivity of an aqueous solution. The conductivity, expressed in millisiemens per meter (ms/m), measures the ability of a solution to allow electric current to pass. This property is directly related to the presence of ions dissolved in water. The higher the content of mineral ions, the better the

electrical conductivity of the solution. Thus, a high conductivity value indicates a significant level of mineralization of the analyzed water. The conductivity meter therefore provides valuable information on the ionic charge contained in an aqueous sample.

- **Operating procedure**
  - Filtration of the stock solution,
  - First make sure that the conductivity meter is correctly calibrated,
  - Turn on the device.
  - Rinse the probe thoroughly with distilled water, then wipe it with a lint-free absorbent paper,
  - Immerse the probe in the harissa sample to be analyzed,
  - Wait for the displayed conductivity value to stabilize.
  - Once the reading has stabilized, note the indicated conductivity value.
  - Between each measurement, repeat the rinsing and drying steps of the probe to avoid contamination.
  - When the analyses are finished, rinse the probe thoroughly with distilled water and keep it dry.


**Figure 30**: Determination of conductivity by conductivity meter **(Personal photo 2024)**.

**NB:** The experiment is repeated four times (4 samples).

### III.5.3.Determination of BRIX degree

✓ **Equipment**

The refractometer is a measuring device that determines the refractive index of the light of a solid or liquid matrix. This index is observed by the deviation of a light beam according to the nature of the medium in which it propagates.The angle of the beam deviates as a function of the rate of soluble dry matter in the medium, the higher the concentration of soluble dry matter, the greater the refraction **[34]**.

✓ **Unit of measurement**

The Brix scale is a unit of measurement used to quantify the content of soluble materials, mainly sugars, present in a liquid. A Brix degree (°Bx) corresponds to the mass percentage of sucrose contained in an aqueous solution.

The unit of measurement degree Brix (°B) owes its name to Adolf F. Brix, a scientist of the nineteenth century. 1°B corresponds to the refractive index of a 1% sucrose solution in water **[35]**.

✓ **Operating procedure**

- Drop a few drops of the sample (product) on the surface of the primary prism. Then close the secondary prism using the button provided for this purpose. Make sure that the sample is very homogeneous and free of air bubbles.
- Open the eyepiece cover and close the mirror.
- Look into the eyepiece and focus on the cross.
- Adjust the ladder window to get the best possible lighting.
- Move the cross using the button until it is positioned at the intersection between the light area and the dark area.
- If necessary, change the color shades using the scatter button.
- Make a final fine adjustment to perfectly position the cross on the light/dark intersection using the appropriate button.
- Note the Brix percentage value indicated on the upper part of the graduated scale.


**Figure 31**: Determination of BRIX by refractometer **(Personal photo 2024)**.

**NB:** The experiment is repeated four times ( 4 samples).

### III.5.4.Determination of titratable acidity

The titratable acidity corresponds to the sum of the free mineral and organic acids in. This is citric acid in the case of harissa. It is determined according to the method described by **(AFNOR, 1974)**.

The titratable acidity of the harissa, expressed in citric acid content per unit volume is determined by titrimetry.

- **Principle**

The principle of the method consists in a titration of the acidity with a hydroxide solution

of Sodium (NaOH) in the presence of Phenolphthalein as a colored indicator.

- **Operating procedure**

Take 10 ml of the sample (filtrat) and pour them into a beaker equipped with a stirrer. Add 0.25 to 0.5ml of Phenolphthalein and, while stirring, pour the sodium hydroxide solution into the burette until a persistent pink coloration is obtained for 30 seconds.

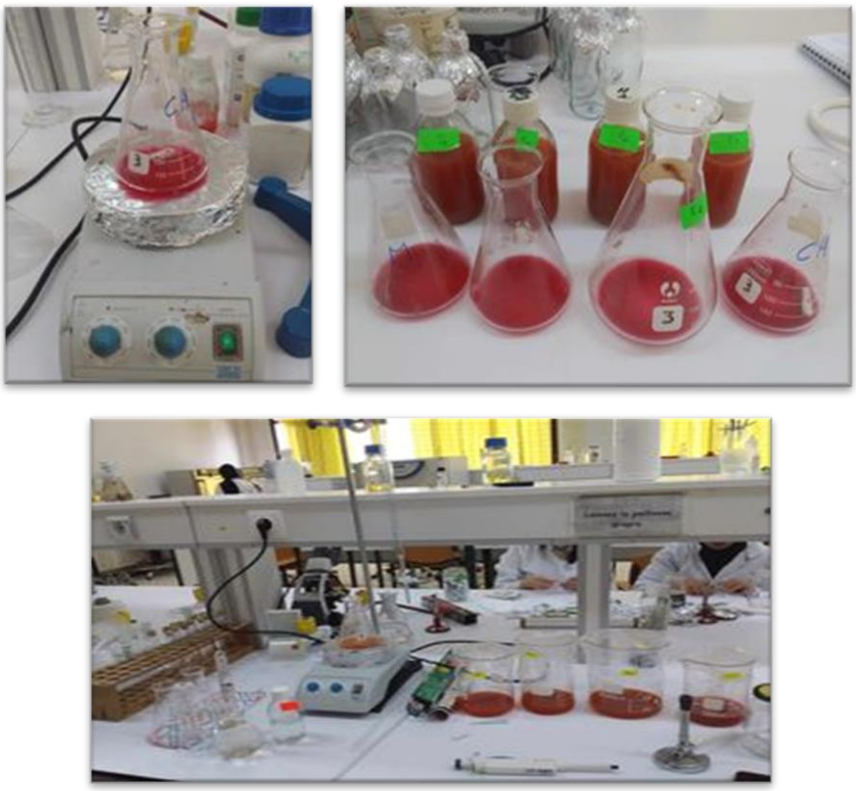


**Figure 32**: Determination of acidity **(Personal photo 2024)**.

Calculates the acidity:

$$C0 = \frac{V1x0.01x1000}{V0}$$

With:

**V0**= volume of harissa (filtrate) in ml of the test sample.

**V1**= volume in ml of the soda solution 0.111N

The acidity is expressed in g/l.

**NB:** The experiment is repeated four times (4 samples).

### III.5.5.Determination of the ash content (AFNOR V76 - 124, 1994 )

The ashes constitute the residue of a fruit or vegetable juice, or of a derivative product,obtained after having completely eliminated their organic substances by calcination and evaporation of water.

*** Principle**

The ash is determined by gravimetric method.

*** Experimental protocol**

2g of each sample to be analyzed were placed in capsules, then placed in the muffle furnace set at 525°C ± 25°C.

For 5 hours until a gray, light or whitish color was obtained. The capsules are removed from the oven and placed in the dryer for cooling.

*** Expression of the results**

The organic matter content, expressed in %, is calculated as follows:

$$MO\% = [(M1 - M2)/P] \times 100$$

With:

**MO%**: organic matter.

**M1**: mass (g) of the capsules + test sample M2: mass (g) of the capsules + ash.

**P**: mass (g) of the test socket.

The ash content (Cd), expressed in %, is calculated as follows:

$$\boldsymbol{Cd}\% = 100 - MO$$



**Figure 33**: Determination of ash contents **(Personal photo 2024)**.

### III.6.Microbiological analyses of harissa concentrate

The primary objective of microbiological analysis for canned food is to highlight or rule out the presence of bacteria or other microorganisms likely to alter the properties of products.

This scientific discipline is dedicated to the study of microorganisms such as bacteria, viruses, yeasts and molds present in various environments, especially food. Its purpose is to detect, identify and characterize these microbes.

For canned goods, this ensures their safety by detecting possible pathogens or undesirable agents. This also makes it possible to control the microbiological quality throughout the production processes, by detecting contaminations.

Bacteriological analyzes are essential to verify the compliance of canned food with hygienic regulations. They evaluate the presence of pathogenic flora and alterations that can contaminate these foods, which are rarely sterile at the source or after handling.

They thus aim to detect and quantify pathogenic microorganisms by enumeration, but also germs that are indicators of hygienic deficiencies. Some are completely banned such as salmonella, while others can be tolerated in limited quantities.

The identification of microbial contaminations, pathogenic or not, is crucial to guarantee the safety of canned food for the consumer.

Microbiological analyzes are an influential means of investigation in terms of the control of the quality and repression of fraud, since they make it possible to reveal the presence or absence of pathogenic microorganisms and/or their toxins **(Guiraud, 1998)**.

### III.6.1.sample preparation

The stock solution is prepared under aseptic conditions from a sample of 25g of product (harissa) and homogenized with 225ml of distilled water. The stock solution is considered to be the $10^{-1}$.


**Figure 34**: Preparation of the stock solution **(Personal photo 2024)**.

### III.6.2. Preparation of decimal dilutions

- **The dilution technique**

The mother suspension is diluted successively in a sterile physiological solution, for example tryptone salt (TS).

The decimal cascade dilution is carried out by transferring a test sample of 1ml of suspension to be diluted in a tube containing 9ml of TS.

For dilutions other than decimal dilution, the amount of TS is adjusted at the test dose.

The number of dilutions necessary is chosen according to the expected concentration of microorganisms in the suspension **[36]**.

The dilutions are carried out in cascades starting from the stock solution $10^{-1}$. They are according to the nature of the product and vary between $10^{-1}$ and $10^{-4}$ in order to facilitate the counting.

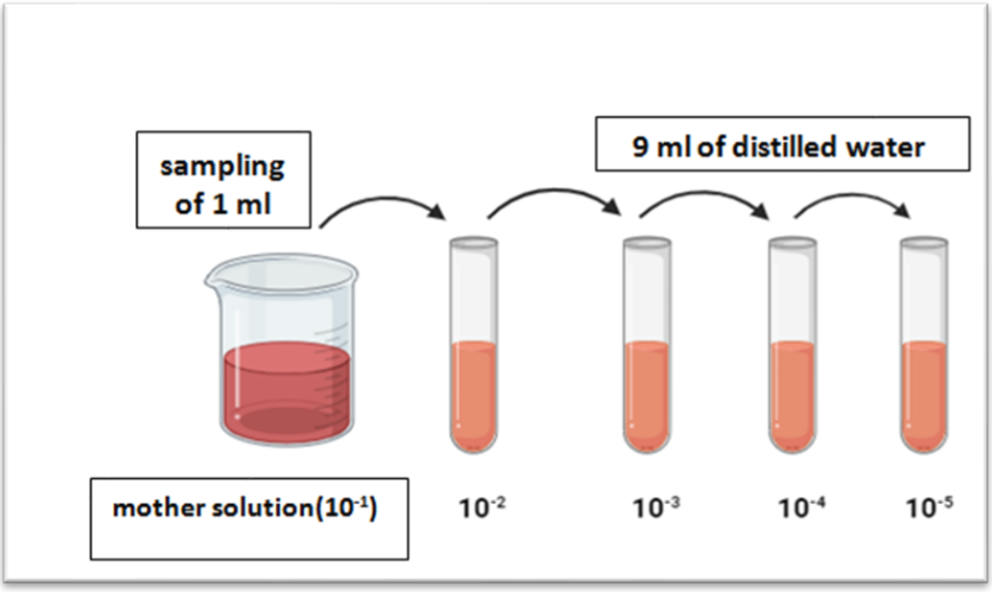



**Figure 35**: Dilution technique **(Personal 2024)**.

## III.7. Microbiological analysis methods

### ❖ Preparation of culture media

The preparation of a culture medium is carried out by simple dissolution of the constituents of the medium in water, optionally heating the mixture, which is reduced by dilution to the final volume 1000mL.

After filtration in certain particular cases and adjustment of the pH, the liquid culture medium is distributed in test tubes or in vials. Sterilization is carried out in an autoclave at 110-120°C. for 20-30min and sometimes under more prolonged conditions. If certain constituents of the medium can be altered by heat, they are sterilized separately in the state of solution by sterile filtration and added to the rest of the medium previously sterilized in an autoclave, with all the appropriate aseptic precautions.

Likewise, some vitamins that would be destroyed by heat, are subjected to sterilizing filtration using special filters, such as millipore filters. Finally, some components such as sugars can react with proteins at the sterilization temperature (Maillard reaction), which causes a browning medium and the appearance of toxic products. The sugars are then sterilized separately of The purity of the crops is essential and any cause of contamination must be discarded **(N.Guezlane_tebibel et *al* ., 2008)**.

**Figure 36:** preparation of the culture medium **(Personal photo 2024)**.

### III.7.1. Search and enumeration of total mesophilic aerobic germs

- **The Definition of mesophile germs**

The total aerobic mesophilic flora is the set of microorganisms capable of forming multiply in the air at medium temperatures, more precisely those whose optimum growth temperature is located between 25 and 40°C. They can be pathogenic microorganisms or alteration.

**(BOUGEOIS and LEVEAU J., 1996)**.

**The operating procedure**

- From the decimal dilutions ($10^{-1}$,$10^{-2}$), aseptically bring an amount of 1ml (20 drops) to the bottom of the empty kneading tins, prepared and numbered in advance for this use.

- Then, mass seeding 1ml of each dilution in the PCA medium previously melted in a water bath and then cooled to 45°C.

-Make circular movements back and forth or eight (8) shape to allow the inoculum to mix with the agar used.

- Let it solidify on the benchtop.

- Incubate the prepared cans with the lids at the bottom, in the incubator at 30°C for 72 h.

**(ISO, 2003)**.

with:

- The First reading at 24 hours.
- The Second reading at 48 hours.
- The Third reading at 72 hours.

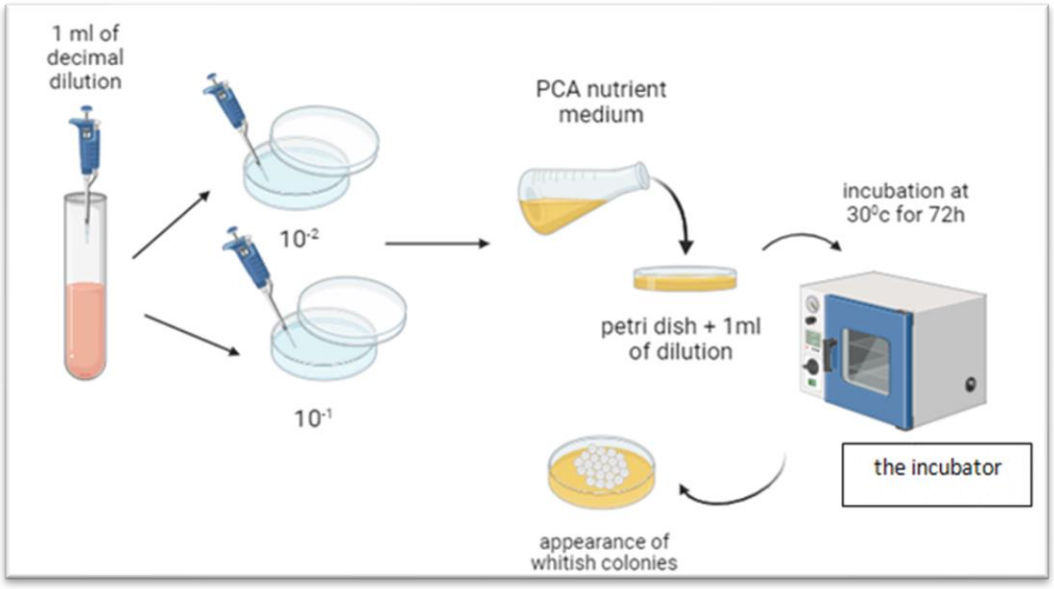



**Figure 37**: Search for total mesophilic aerobic germs **(Personal 2024)**.

The number of microorganisms is calculated according to the following formula:

$$\textbf{number of germs} = \boldsymbol{\Sigma c \,/\, Vx\ (n1 + 0.1n2)\ x\ D}$$

- **Formula N**: number of CFUs per gram or per ml of initial product;
- **Σ colonies**: Sum of the colonies of the interpretable boxes;
- **V (ml)**: volume of deposited solution (1 ml);
- **n1**: number of cans considered at the first dilution retained;
- **n2**: number of cans considered at the second dilution retained;
- **D**: factor of the first dilution retained.

- **Reading**

After the specified incubation period, determine which boxes contain colonies of the lenticular forms. If we notice a rapid invasion of colonies in boxes, count the colonies after 24 hours, then again up to 72 hours **(NF V 08-011)**.

### III.7.2. Research and enumeration of yeasts and molds

- **Yeasts**

Yeasts are microorganisms that have the ability to form alcohol, ethanol, and $CO_2$ (carbon dioxide). They play a role in the formation of aromas. The presence of yeasts on the surface of the yogurts are the index of a pollution which depreciates the appearance and the taste of the products **(Romain et *al*., 2015)**.

- **Molds**

Molds are filamentous fungi developing by a system of branched filaments called thallus or hyphae, which produce spores disseminated by air and water. Molds grow on acidic media. These are weathering microorganisms, widely used in cheese making. The main interesting genera in dairy are: Alternaria **(Isabelle, 2002; Afssa, 2009)**.

All yeasts and molds are detected and counted in food products, pharmaceutical and cosmetic products as well as in the hospital or industrial environment Intermedius **(Delarras C, 2014)**.

The main culture medium used in the research and enumeration of yeasts, molds and other fungi is oxytetracycline glucose agar (OGA) **(Delarras C, 2014)**.

- **The principle**

- **The Inoculation**

In a petri dish containing OGA agar, transfer 4 drops of the first decimal dilution ($10^{-1}$),($10^{-2}$) using a sterile pipette, proceed in the same way with the following dilutions using a new sterile pipette with each decimal dilution.

-Spread the liquid on the surface of the agar plate with a sterile rake pipette.

- **The Incubation**

Incubate the prepared cans with the lids down at room temperature for three to five days **(Delarras C, 2014)**.

- **Reading**

The enumeration is done for the yeast colonies apart and the mold colonies apart.

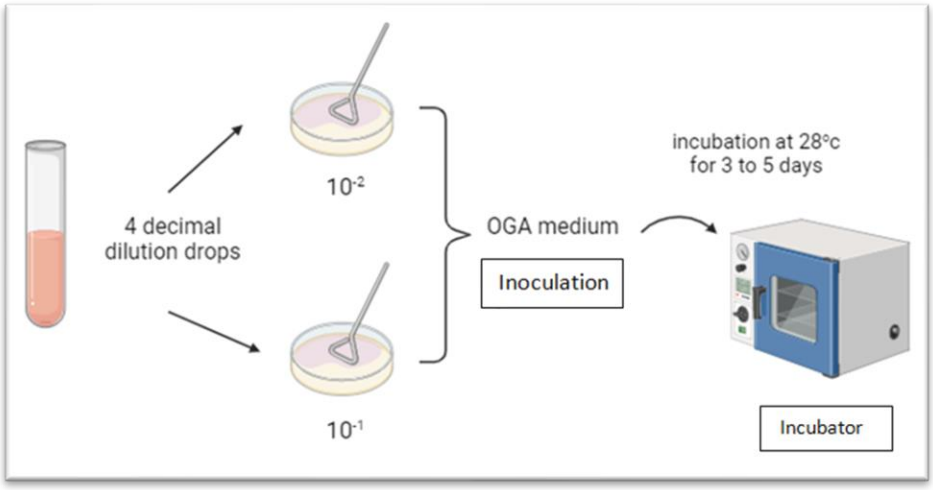



**Figure 38:** Search for yeasts and molds (**Personal** 2024).

## III.7.3.Research and enumeration of total and fecal coliforms

Coliforms are gram-, non-sporulated, oxidase-, aero-anaerobic or facultative anaerobic bacilli, they can develop in the presence of bile salts or other equivalent surfactants, they ferment lactose with acid and gas production in 48 hours at a temperature of 35°C to 37°C (± 0.5°C) **(Delarras C, 2014)**.

The main culture medium used in the research of coliforms in food products is VRBL agar (violet red bile lactose) is used for the enumeration of coliforms and thermotolerant coliforms in food products **(Delarras C, 2014)**.

- **Procedure**

An agar medium (VRBL) is used for the search and enumeration of total and fecal coliforms.The simultaneous presence of purple crystal and bile salts ensures the inhibition of gram-positive bacteria.

- ✓ From the decimal dilutions, aseptically bring an amount of 1ml (20 drops) to the bottom of the empty and sterile kneading tins, prepared and numbered in advance.this operation must be carried out in double for each dilution for the search for :

Total coliforms at 37°C for 24 hours.

Fecal coliforms at 44°C for 24 hours.

- ✓ Complete these boxes with a quantity of about 20 to 15ml of VRBL agar melted and then cooled to 45°C.
- ✓ Then maintain a delicate stirring using circular back-and-forth movements in the shape of 8. Mix the agar well with the inoculum.-leave the medium on the bench for 1minute to solidify, then pour about 5ml of the same agar again , to avoid all kinds of contamination.

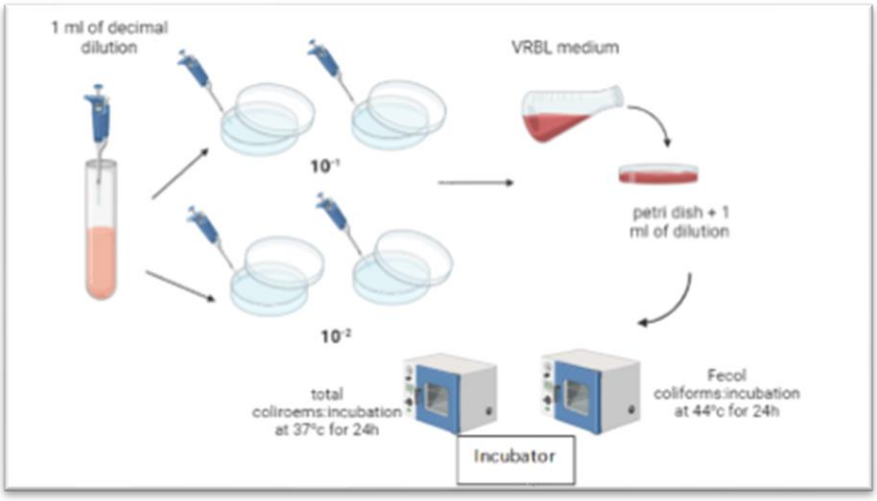



**Figure 39**: Research protocol and enumeration of coliforms **(Personal 2024)**.

- **Reading**

Total and fecal coliforms occur in the form of colonies of bright dark red color **(Boudjir and Zehar., 2019)**.

### III.7.4. Search for fecal streptococci

- ❖ **Search and enumeration of fecal Streptococci**

Under the general name of Enterococci, the fecal Streptococci are in much of it man-made. Some bacteria in this group come from the feces of animals such as: *Streptococcus bovis, S. equinus, S. gallolyticus, S. alactolyticus*, or even found on plants.

They are spherical bacteria, in pairs or in chains, Gram-positive, catalase-negative, facultative anaerobes that hydrolyze esculin in the presence of bile However, they are considered as indicators of a fecal pollution of which their main interest is the resistance to desiccation and persist longer in the water. The presence of fecal streptococci is assessed by enumeration in a liquid medium using two culture broths (Rothe medium and the middle Eva Litsky) as follows (figure 40) **(Clausen et *al.*, 1977 ; Farrow et *al.*, 1984 ; Bitton, 1999 ; CEAEQ, 2006).**

❖ **the operating procedure**

- Their research is carried out on the Rothe medium distributed in test tubes (fig.40).
- From the dilutions, wear aseptically :
    - 3 times 10ml in 3 tubes containing 10ml of Rothe D/C medium.
    - 3 times 1ml in 3 tubes containing 10ml of Rothe S/C medium.
    - 3 tubes containing 10ml of Rothe S/C medium.
- Mix well and the inoculum then incubate at a temperature of 37°C for 24 to 48 hours.
- The test is noted positive when there is an appearance of a microbial disorder in the Rothe medium.
- Confirmation test if the presumption test is positive, transplanting on Eva Litsky medium is performed.
- The incubation of the tubes is carried out at 37°C for 24 hours.

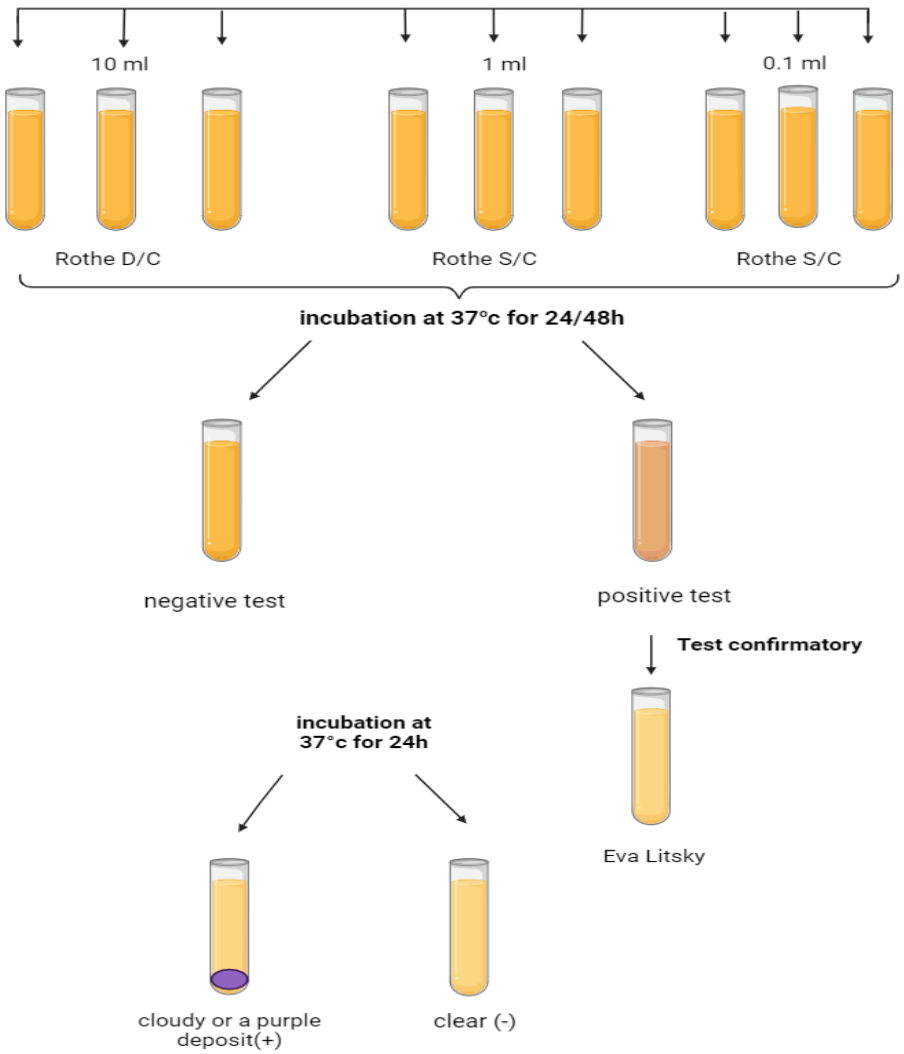



**Figure 40**: Search and enumeration of fecal Streptococci **(Personal 2024)**.

❖ **Reading**

The positive test results in microbial disorder is a purple (whitish) pellet; there is at least presence of a fecal streptococcus.

### III. 7. 5. Research and enumeration of anaerobic sulfito-reducing bacteria (ASR)

Sulfito-reducing anaerobes (ASR) are in the form of Gram+ bacteria, developing in 24 to 48 hours on a liver meat agar (VF) by giving colonies by reducing the sodium sulfite ($Na_2SO_3$) which is found in the medium, to a sulfide which in the presence of $Fe^{2+}$ gives FeS (iron sulfide) of black color. The spores of ASR generally constitute indications of old contamination **(Lebres, 2008)**.

- **The operating procedure**

✓ From the decimal dilutions, introduce 25ml into a sterile tube, place it in a water bath at 80°C for 10minutes, the purpose of which is to destroy the vegetative forms of these bacteria possibly present.

✓ After heating, immediately cool the tube under tap water.

✓ Then distribute the contents of this tube, in 4 different and sterile tubes, at a rate of 5 ml per tube.

✓ Add about 18 to 20ml of agar to the melted liver meat and then cool to 45 ± 1°C, followed by the addition of an amount of 0.5 ml of the sodium sulfite solution and 4 drops of the iron alum solution.

✓ Gently mix the medium and the inoculum, avoiding the introduction of air bubbles. (Avoid the introduction of oxygen). Then paraffin oil is added to create anaerobic conditions.

✓ Leave to solidify on a bench for about 30 minutes, then incubate at 37°C for 24 to 48 hours **(Rodier, 2005 ; Guiraud, 1998).**

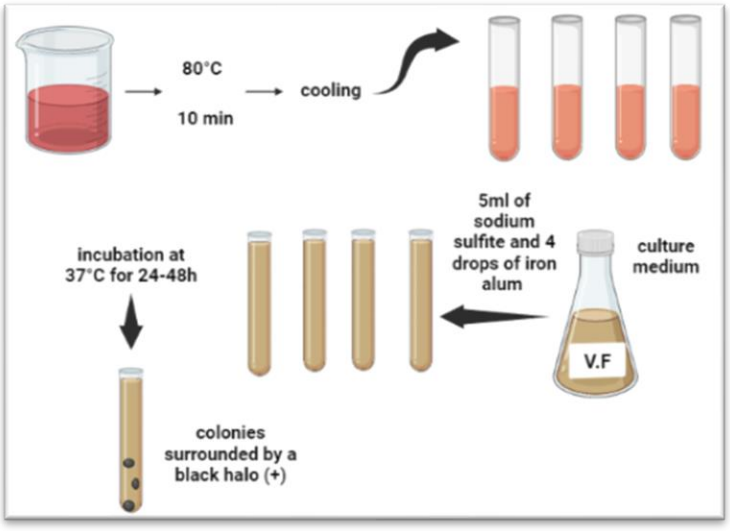



**Figure 41**: Research and enumeration of anaerobic sulfito-reducing bacteria (ASR) **(Personal 2024)**.

- **Reading**

Counting is carried out for any black colony surrounded by a black halo expressed in the number of spores.

**III.7.6. Search for pathogenic germs**

There is a wide variety of pathogenic or potentially pathogenic bacteria (opportunistic) for humans in all types of waters. These live or survive in the environment, either from human discharges, eliminated by sick subjects or healthy carriers, either being indigenous and able to adapt to humans **(Rodier, 2009).**

The desired germs are chosen, within the limits of the available means. The germs sought are:Salmonella, Staphylococcus aureus, Shigella, Pseudomonas aeruginosa. The media used are: Mac Conkey, Salmonella-Shigella Agar (SS), Hektoen, Chapman, Cetrimide, King A, and King B.

1. **Research of salmonella**

Salmonella are intestinal parasitic bacteria of vertebrates, agents of salmonellosis **(Delarras C, 2014).**

Salmonella are gram-bacilli, most often mobile, are oxidase-, ferment glucose with or without gas production, they reduce nitrates to nitrites with exceptions **(Delarras C, 2014)**.

The Solmonella are divided into two large groups: the minor and the major which are highly pathogenic **(Pechère et *al*., 1982; Carbonnelle, 1988 ; Larbres et *al*., 2008)**.

The SS (Salmonella-Shigella) agar medium is a selective and differential culture medium used for the isolation and identification of pathogenic bacteria belonging to the genera Salmonella and Shigella, which are part of the Enterobacteria family **(Delarras C, 2014)**.

- **The operating procedure**
- **Isolation of salmonella**

From the decimal dilution $10^{-2}$,$10^{-3}$ The seeding is carried out by streaking with a pasteur pipette after having poured the Salmonella-Shigella agar (S-S), then incubated at 37°C for 24 hours .

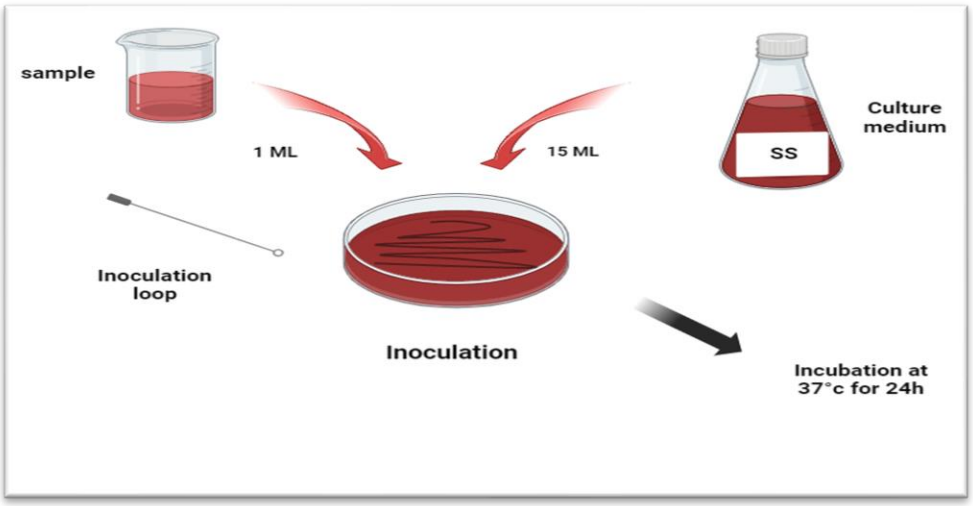



**Figure 42**: Isolation of salmonella **(Personal 2024)**.

## 2. The search for staphylococci

Staphylococci are Gram-positive cocci, are ubiquitous germs that can live as saprophytic bacteria in nature (soil, air, water, food ...), commensal bacteria on the skin and mucous membranes of humans and animals (mammals), . The genus Staphylococcus consists of several species, the main ones being: *Staphylococcus aureus, Staphylococcus epidermis, Staphylococcus saprophyticus, Staphylococcus Intermedius* **(Delarras C, 2014)**.

- **The Chapman medium**

The Chapman medium is characterized by its high concentration of sodium chloride, which allows selective isolation of staphylococcus.

The fermentation of mannitol is indicated by the turn to yellow of the colored indicator (the phenol red) around the colonies **(Rodier, 2009)**.

- **The operating procedure**

Starting from a decimal dilution $10^{-2}$, $10^{-3}$ and using a sterile pasteur pipette, take 2 drops and inoculate on a can of Chapman medium. Incubate at 37°C for 24 hours (fig.42) **(Delarras C, 2014)**.

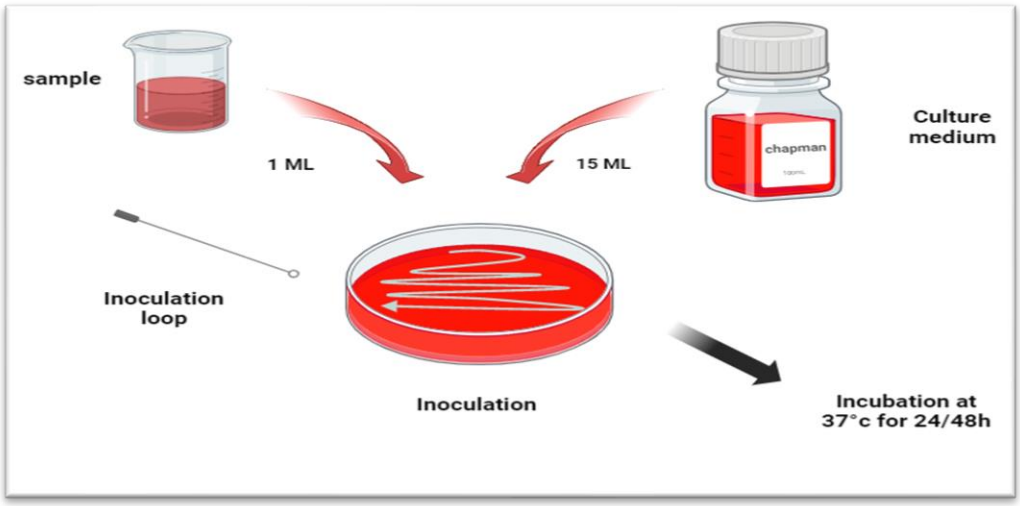



**Figure 42**: The search for staphylococci **(Personal 2024)**.

- **Reading**

- The colonies of Staphylococcus aureus are surrounded by a yellow halo due to the attack of mannitol.

- The Chapman medium only allows an orientation for the identification of the species *Staphylococcus aureus*. But this is only a presumption step and confirmation by specific tests remains mandatory.

**(Joffin and Leyrol, 2001)**.

1. **The Search for Pseudomonas**

Pseudomonas is understood to mean a bacterium which is in the form of a gram-negative bacillus possessing the oxidase enzyme and capable of producing ammonia from acetamide and of not degrading lactose **(Rejsek, 2002)**.

- **Cetrimide**

Cetrimide is a selective medium for the isolation and enumeration of pseudomonas aeroginosa in biological products, water, food, pharmaceutical and cosmetic products **(Delarras C, 2014)**.

- **The operating procedure**

Starting from a decimal dilution $10^{-2}$,$10^{-3}$ decimal dilution and using a sterile pasteur pipette, aseptically bring 2 drops and inoculate on the surface with cetrimide agar, then incubate them at 36 ± 2 °C for 18 to 24 h.

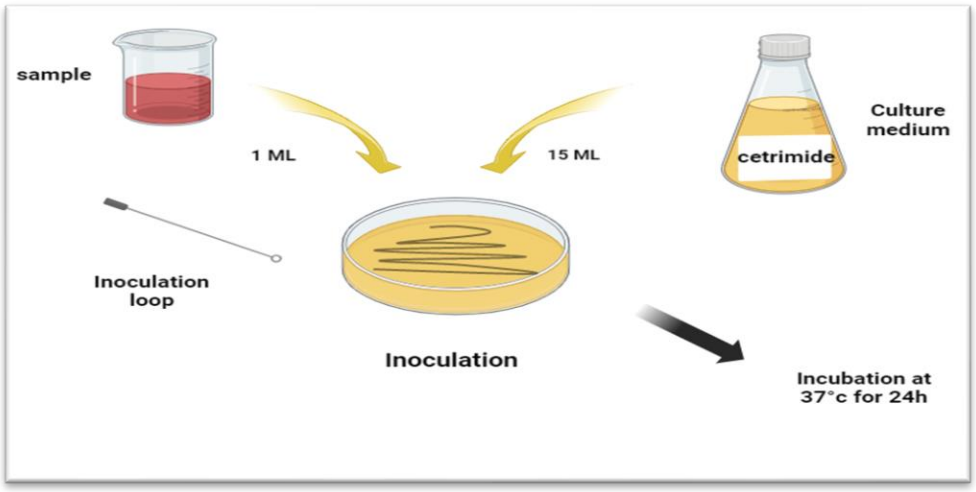



**Figure 43**: The Search for Pseudomonas **(Personal 2024)**.

- **Reading**

Considered as characteristic colony any colony exhibiting fluorescence, due to the selectivity of the Cetrimide medium. Colonies of Pseudomonas often appear large sizes (1-3mm), with irregular edges, regular smooth and domed **(Hadji F, 2020)**.

- **Identification**

On the King A medium, the search is made for pyocyanin, a blue pigment characteristic of Pseudomonas responsible for the intense blue hue of the culture media. While the search for pyoverdin is done on King B **(Hadji F, 2020)**.

1. **The Search for Shigelles**

All Shigella (genus Shigella) are Enterobacteriaceae found exclusively in humans. They are not part of any commensal flora in humans, they are all pathogenic and specific to the digestive tract eliminated by feces and dispersed in soils and waters where they only survive for a short time. Morphologically, they are Gram-negative bacilli, immobile; devoid of spores and capsules very close to E. coli **(Berche et *al.*, 1988)**.

- **The Cultur**

Starting from $10^{-2}$, $10^{-3}$ decimal dilution, aseptically carry 2 drops and spread it on the surface of Mac Conkey Agar, Salmonella-Shigella Agar (SS) and Hektoen Agar, then incubate them at 36 ± 2°C for 18 to 24 h **(Delarras C, 2014)**.

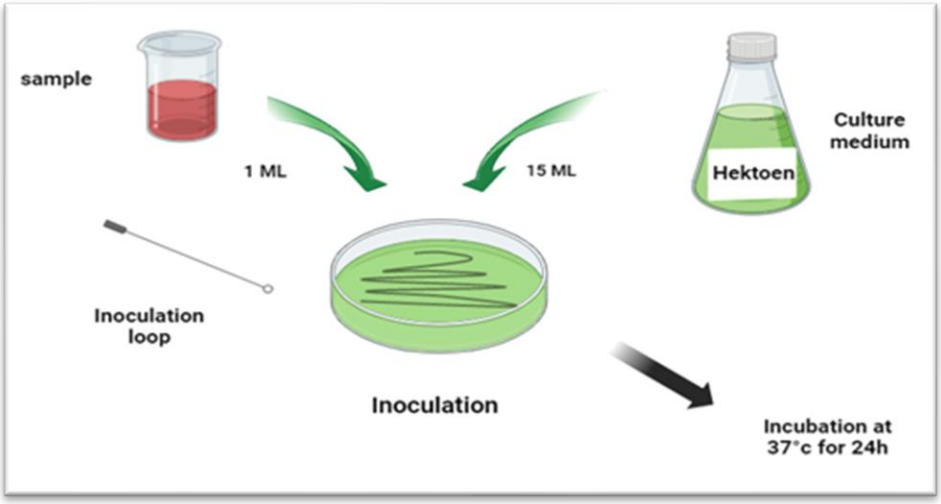



**Figure 44**: The Search for Shigelles **(Personal 2024)**.

### III.8. Biochemical identifications of germs

The identification is based essentially on :

1. Oxidase test.
2. Catalase test.
3. API Galleries.

### III.8.1. Search for oxidase and catalase

**1. oxidase Search**

The term oxidase designates an enzyme sought after in systematic bacteriology. The presence of oxidases would be linked to that in the respiratory chain of the enzyme complex IV: cytochrome-oxidase **(Carbonnelle and Kouyoumdjian ., 1998)**.

This test is the basis for the identification of Gram (-) bacteria and makes it possible to highlight an enzyme: phenyl diamine oxidase of bacteria from their culture in agar medium. This enzyme is capable of oxidizing a reagent: N-dimethyl-para-phenylenediamine **(Abdellioui et *al*., 2012)**.

- **According to The principle**

The oxidase test is based on the bacterial production of an intracellular oxidase enzyme.

In the presence of atmospheric oxygen and cytochrome C, this enzyme oxidizes the reagent to form a purple-colored compound, indophenol **(Delarras C, 2014)**.

- **The operating procedure**

On a clean and sterile slide, deposit an oxidase disc then prepare a bacterial suspension from the desired colony and deposit a drop of the suspension on the disc **(Bara, 2016)**.

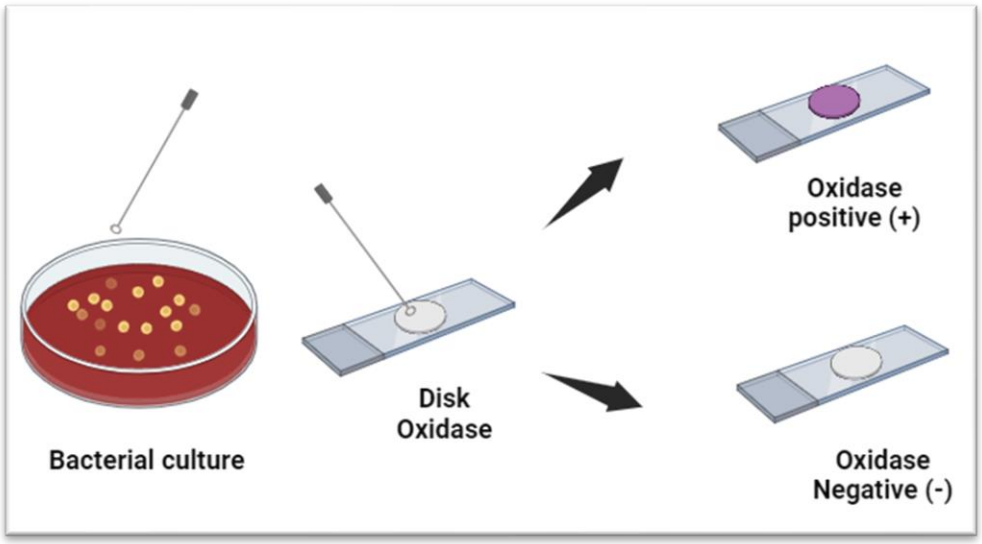



**Figure 45**: Oxidase Search **(Personal 2024)**.

❖ **The technique**

The reagent can be found in two forms :

o Either in solution: place a square of filter paper on a glass slide and soak it with a freshly prepared solution of reagent.

o Either in the form of a disc pre-impregnated with the reagent. In both cases, crush with a tap of a pasteur pipette a colony of germs to be studied on this paper (instrument not oxidizing the reagent) (figure.45) **(Amira W, 2008)**.

❖ **Reading**

o If the colony takes on a purple hue the germ has an oxidase, the test is positive.

o If the colony remains colorless the germ does not have oxidase, the test is negative **(Delarras C, 2014)**.

**2.Catalase search**

Catalase is a metabolic product that is toxic to bacteria. To do this, a drop of 30% hydrogen peroxide ($H_2O_2$) is added to the colony placed on a microscope slide. A bubble production (gas release) is noticed when the reaction is positive. This test is used in particular to differentiate the bacteria of the family of the Micrococaceae (*Staphylococcus*) catalase (+) from that of the (*Streptococcaceae*) catalase (-) **(Delarras C, 2003)**.

**According to the principle**

This enzyme allows the degradation of hydrogen peroxide to water and free oxygen which is released in gaseous form according to the following reaction: **(Delarras, 2014)**.

$H_2O_2 \Longrightarrow H_2O + 1/2\ O_2$

This test is the basis for the identification of Gram (+) bacteria **(Hocine et *al*., 2022).**

- **The technique**

On an object-carrying slide, we deposited a drop of hydrogen peroxide ($H_2O_2$) and we added a dose of bacteria taken from the agar medium of the strain (fig 47) **(Hocine et *al*., 2022)**.

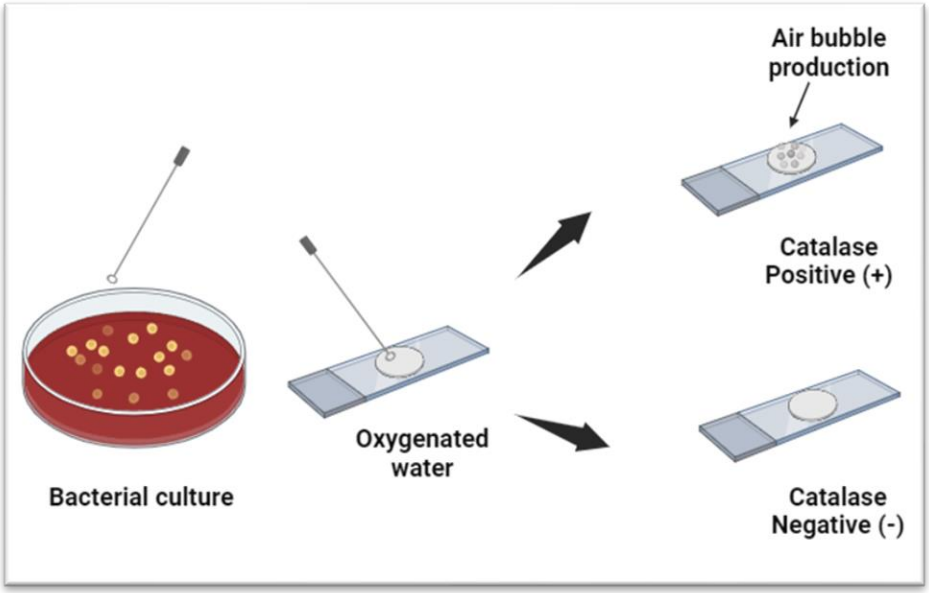



**Figure 46**: Catalase search **(Personal 2024)**.

- **Reading**

If a release of gas bubbles (oxygen) appears, the so-called positive test **(Delarras C, 2003).**

### III.8.2. API Galleries

They are marketed in boxes of 25 galleries always containing a complete technical sheet (on paper) which generally presents the same sections; however, particularities may exist for this or that gallery **(Delarras C, 2014)**.

- Several API galleries can be used for the identification of enterobacteria :

**a. API Gallery 20 E**

The API gallery marketed, is a standardized system for the identification of bacteria it is composed of a variable number of micro tubes (10 or 20 most often), containing dehydrated substrates which make it possible to carry out biochemical tests **(Camille , 2007)**.

- **Inoculation of the gallery**

From a fresh culture on agar medium, a dense bacterial suspension is prepared by dissociating 4-5 colonies in 5ml of sterile physiological saline water. The micro tubes are carefully filled with this suspension using a micropipette. The filling of the micro tube is

carried out avoiding the formation of air bubbles which would prevent contact between the bacteria to be identified and the reagent or substrate to be tested **(Kari and Laifaoui., 2013)**.

The cups were filled taking care to create a horizontal or slightly convex level for the three CIT, VP and GEL tests. Incompletely filled or overfilled cups can lead to incorrect results. In order to create the anaerobic conditions required for biochemical tests of the transformation of the amino acids arginine, lysine and ornithine, respectively, by the Enzymes ADH, LDC and ODC. The release of ammonia from urea thanks to the presence of the enzyme ammonia urease (urea) and the production of $H_2S$. The corresponding cups. Have been covered with vaseline oil **(Kari and Laifaoui., 2013).**

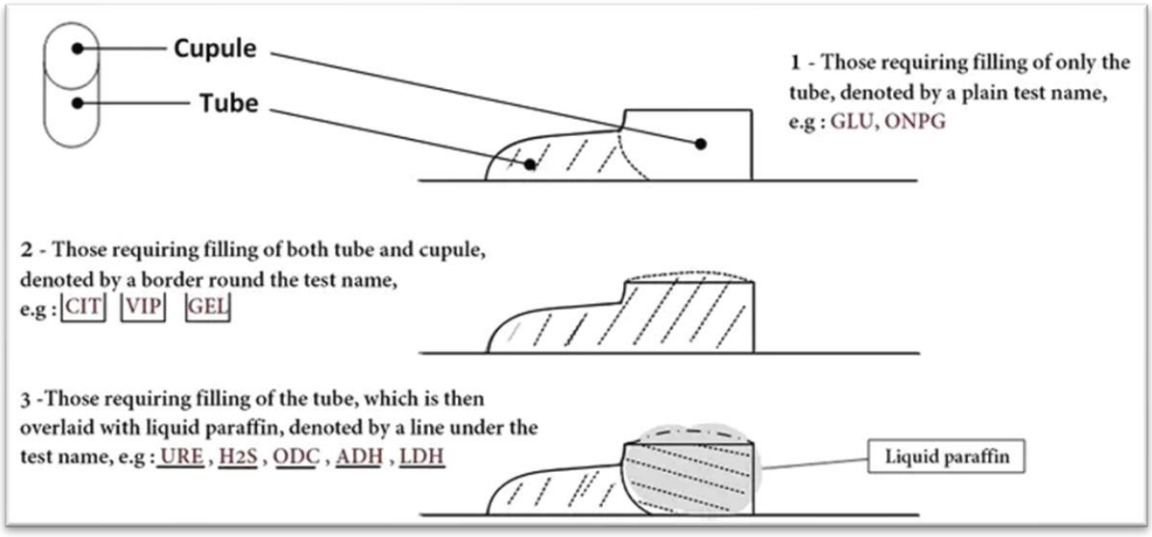



**Figure 47**: Preparation of the API 20TH gallery **[37]**.

- **Gallery reading**

After incubation at 37°C, for 24 h, the results of all the spontaneous reactions are noted on the card, then the tests requiring the addition of reagents (TDA, VP and Kovacs) are revealed. The identification is then obtained using an identification software. The reading of these reactions is done using the reading table **(Kari and Laifaoui, 2013)**.

**Table 10:** The reagents to be added to API 20E.

| wells | reagents |
|---|---|
| **TDA** | **a drop of TDA reagent** |
| **IND** | **a drop of reagent james or kovacs** |
| **VP** | **a drop of reagent VP1 then VP2** |

The reading of these reactions (positive or negative) is done according to the color variations (fig:48).

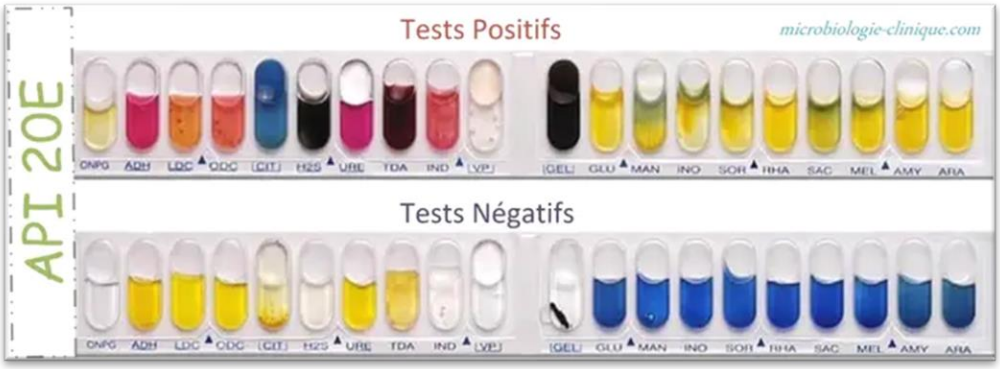



**Figure 48**: API 20E positive vs negative **[37]**.

**b. API Gallery 20 NE**

❖ **Principle**

The API 20NE gallery is a miniaturized and standardized version of conventional biochemical techniques for the identification of Gram-negative bacteria. The API 20NE gallery comprises 20 micro tubes containing substrates in dehydrated form, these micro tubes are inoculated with a bacterial suspension which reconstitutes the media. The reactions produced during the incubation period result in spon tan colored turns where reveal by the addition of the reagents **(Bukhatem, 2013)**.

❖ **Technical**

**Preparing the gallery**

✓ Put sterile distilled water on the bottom of the box (honeycomb part);

✓ Place the gallery on the bottom of the box;

✓ Cover the box with its lid;

✓ Write the strain reference on the side tab of the box.

**Preparation of the inoculum**

✓ Open an ampoule of API Na Cl 0.85% and using a sterile Pasteur pipette, Take a single well-isolated colony on agar medium;

✓ Make a bacterial suspension by homogenizing carefully.

**Inoculation of the gallery**

✓ Introduce the bacterial suspension into the micro tube of the gallery using a sterile pipette:

✓ Close the incubation box again, incubated at 37°C for 24 hours **(Bukhatem , 2013)**.

❖ **Reading**

The reading of these reactions is done using the reading table and the identification of the strains is obtained using the identification table of the analytical catalog **(Bukhatem, 2013)**.

**Table 11:** The reagents to be added to the API 20 NE.

| wells | reagents |
|---|---|
| **$NO_3$** | **a drop of reagent NIT1 then NIT2** |
| **TRP** | **a drop of reagent JAMES** |

❖ **Assimilation tests**

- Observe the bacterial growth. A cloudy cup indicates a positive reaction, shoots of intermediate, intensity can be observed and noted F or ±,
- Once this reading has been carried out, re-identification must be practiced as indicated in the paragraph "interpretation".
- Re-incubation is necessary in the following cases,
- Low discrimination,
- Unacceptable profile or dubious profile.

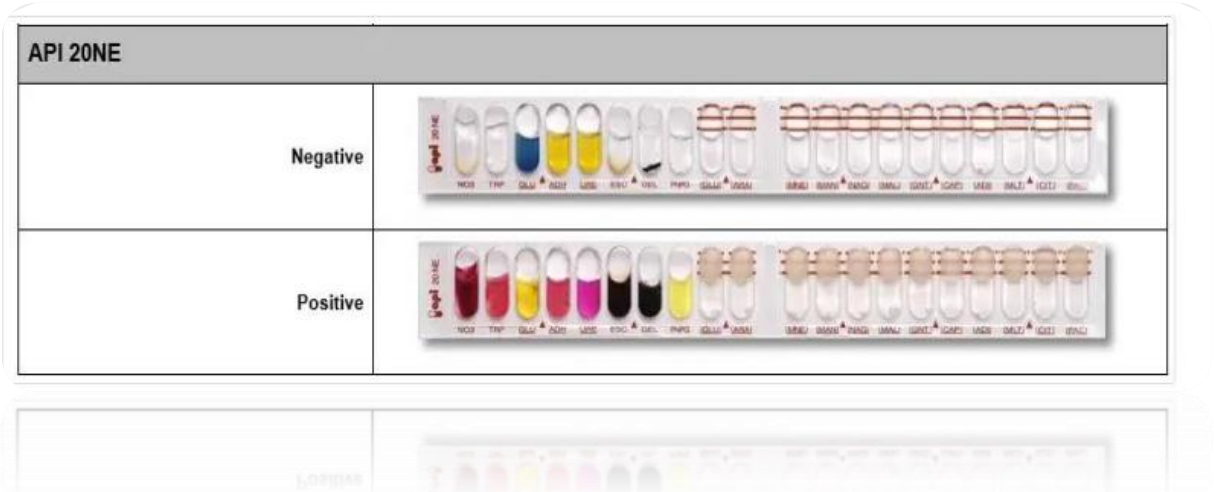


**Figure 49**: API 20NE positive vs negative **[37]**.

### c. API Staph Gallery

API Staph is a standardized system for the identification of the genera Staphylococcus, Micrococcus and Kocuria including miniaturized biochemical tests as well as a database. The complete list of bacteria that can be identified with this system is present in the identification table at the end of the leaflet **(baghdad et *al*., 2023)**.

✓ **Inoculation of the gallery**

- Using a pipette, fill the tubes of the gallery. Fill only the tubes and not the cups, without exceeding the level of the tube. To avoid the formation of bubbles at the bottom of the tubes, place the tip of the pipette on the side of the cup, tilting the incubation box slightly forward.
- Create an anaerobiosis in the ADH and URE tests by filling their cup with paraffin oil to form a convex meniscus.
- Incubate at 36°C ± 2°C for 18-24 hours.
- After incubation, read the reactions in accordance with the reading table by adding 1 drop of each of the following reagents :

**Table 12**: The reagents to be added to the Staph API.

| wells | Reagents |
|---|---|
| **VP** | **one drop of reagent VP1 then VP2** |
| **NIT** | **one drop of reagent NIT1 then NIT2** |
| **PAL** | **one drop of reagent ZYM A and ZYM B** |

**Figure 50** : API Staph positive vs negative **[37]**.

# Chapter IV

# Results and Discussion

This chapter is dedicated to the exhaustive presentation of the observations and results obtained during the study carried out on the harissa product.

All the data relating to the measured physicochemical parameters as well as the microbiological parameters of the harissa samples analyzed are exposed. These results are transcribed in the form of synthetic tables and graphical representations (diagrams) making it possible to easily visualize the various variations observed for all the parameters studied.

This detailed presentation of the results obtained, both in numerical and illustrated form, aims to clearly and structurally restore all the observations made on the harissa product as part of this study.

## IV.1.Results of physico-chemical parameters

The parameters controlled are: Brix, pH, conductivity, acidity. The results obtained are compared with the standards of the cannery.

Note that the samples contain different amounts of olive oil:

T0: 0 ml, T1:10 ml , T2: 15ml, T3:20 ml .

**NB: The witness cans does not contain any amount of olive oil for the entire period of the experiment.**

### IV.1.1.Determination of pH

The pH is an indicator of biological and chemical quality, its measured gives us help on the quality of the product to be analyzed **(NF v 05-108.1970)**.

The measurement of the pH plays a primary role in the evaluation of the harissa. Beyond its influence on the organoleptic qualities of the product, the pH constitutes a key indicator to determine the suitability for food preservation.

This parameter is therefore of paramount importance in the analysis of the harissa. The pH measurement tests are essential to decide on the marketing or not of the different batches of harissa concentrates produced.

As part of this study, pH readings were taken on 4 separate samples, at room temperature. The results obtained for each sample are recorded in the following table:

**Table 13**: The pH values of harissa studied.

| Samples | $T_0$ =0 days | $T_1$ =7 days | $T_2$ =15 days | $T_3$ =30 days | standard |
|---|---|---|---|---|---|
| **witness** | 3.82 | 3.72 | 3.68 | 3.60 | |
| **Sample 1** | 3.85 | 3.71 | 3.67 | 3.64 | ≤ 4.5 |
| **Sample 2** | 4.12 | 4.09 | 4.05 | 4.04 | |
| **Sample 3** | 3.91 | 3.71 | 3.69 | 3.67 | |

These measured pH values will make it possible to evaluate the conformity of the harissa samples analyzed with regard to the quality and conservation requirements of the product.

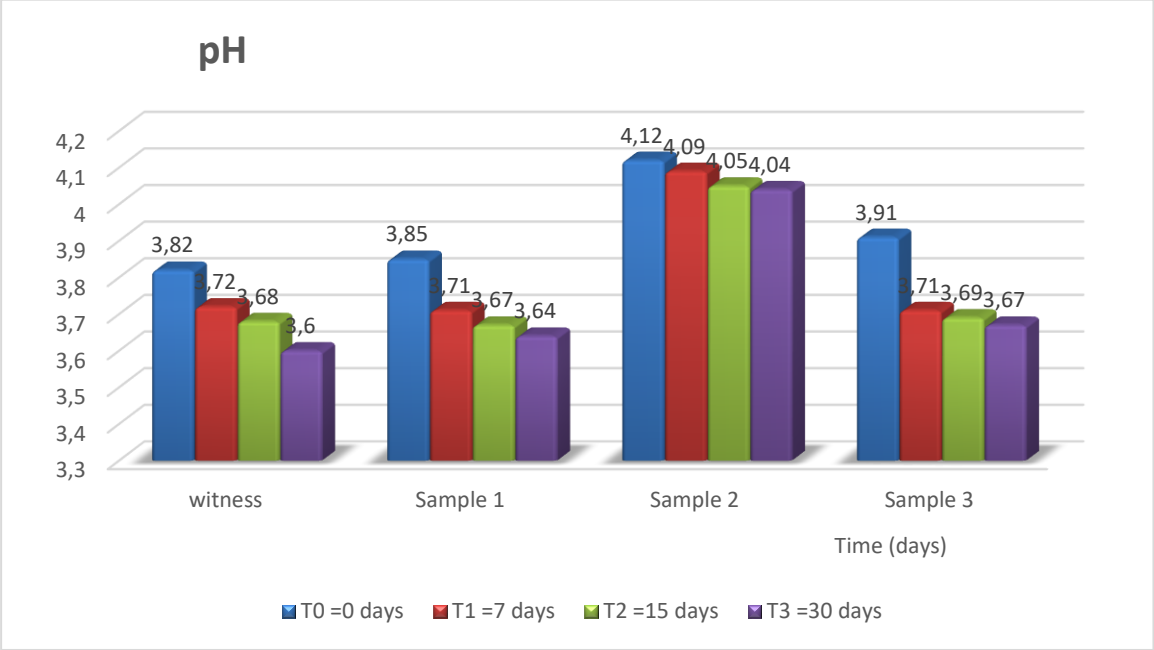



**Figure 51**: Histogram of the pH of the analyzed samples as a function of time.

According to the results, in the figure 51 the pH values of the analyzed samples (controls, sample1, sample2 and sample3) are between 3.60 and 4.12. Overall, the pH of the 4$^{th}$ sample remained acidic from the first day the cans were opened until the 30th day.

The pH values remained practically constant in the 2 cases with or without the addition of olive oil, and they correspond to the standard of **(codex alimentarius)** fixed at 4.5.

The addition of this oil to the harissa in different proportions accompanies a contribution of fatty acids and therefore a decrease in pH and increase in free acidity. The pH concentrated harissa acid provides it with a certain pleasant taste and can exercise some protection against any attack by acid-sensitive bacteria. **(BOUHADID et *al.*, 2022)**.

### IV.1.2. Determination of conductivity

Conductivity is the measure of the ability of a water to conduct an electric current. The conductivity varies depending on the temperature. It is related to concentration and nature dissolved substances. In general, mineral salts are good conductors as opposed to the organic matter that drives little **[38]**.

The results obtained for each sample are recorded in the following table:

**Table 14**: The Conductivity values of harissa studied.

| Samples | $T_0$ =0 days | $T_1$ =7 days | $T_2$ =15 days | $T_3$ =30 days |
|---|---|---|---|---|
| **witness** | 5.26 ms/cm | 4.96 ms/cm | 4.88 ms/cm | 4.79 ms/cm |
| **Sample 1** | 5.38 ms/cm | 4.42 ms/cm | 4.41 ms/cm | 4.40 ms/cm |
| **Sample 2** | 4.29 ms/cm | 4.10 ms/cm | 3.90 ms/cm | 3.90 ms/cm |
| **Sample 3** | 4.84 ms/cm | 4.21 ms/cm | 4.20 ms/cm | 4.19 ms/cm |

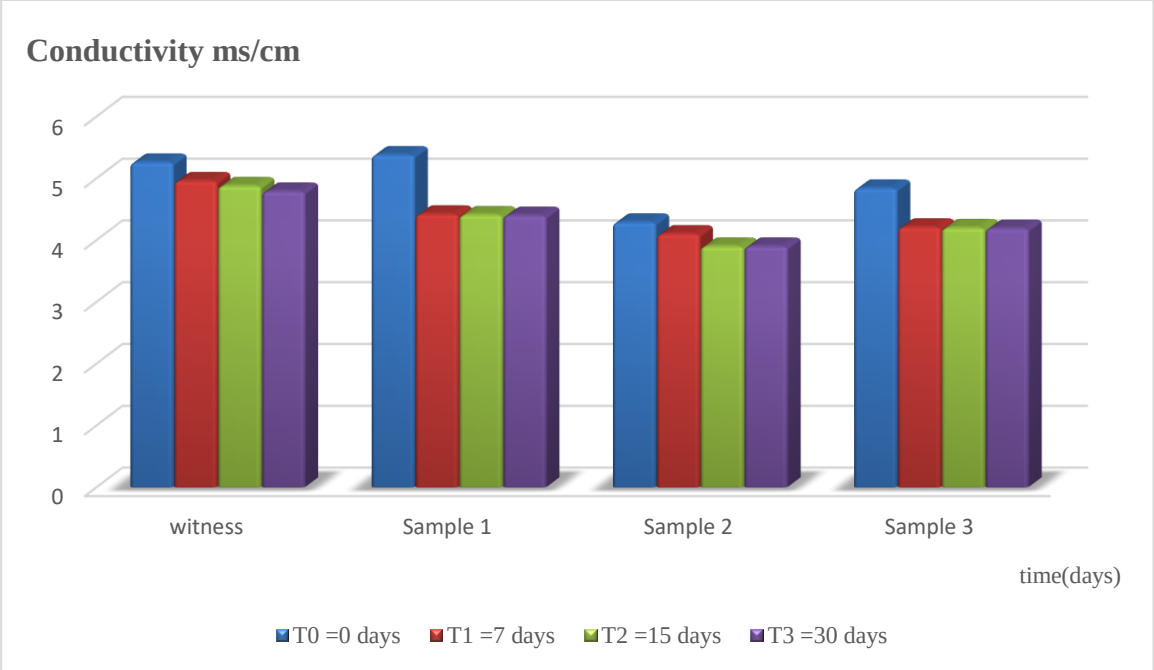



**Figure 52**: Histogram of the Conductivity of the analyzed samples as a function of time.

According to the results, in the figure 52, the conductivity values of the analyzed samples (witness, sample1, sample2, sample3) are between 3.90 and 5.38 from the opening day of the cans up to 30 days. Where we notice a slight decrease in degrees. In both cases, with or without the addition of olive oil.

The pH (hydrogen potential) has a significant effect on the electrical conductivity of an aqueous solution. In an acidic solution, there is a high concentration of hydrogen ions ($H^+$) which increases the conductivity **(Journal of Applied Chemistry, 2013)**.

According to a study published in the **(Journal of Food Engineering 2006)**, Olive oil, being a non-polar and non-ionic substance, has practically no effect on the electrical conductivity of an aqueous solution. However, when olive oil is added to an aqueous solution containing ions, it can slightly change the conductivity due to certain factors. Adding olive oil to an aqueous solution can lead to a slight decrease in electrical conductivity. The authors explain that "olive oil can form a protective layer around water droplets, thus preventing ion mobility and reducing conductivity".

However, this decrease in conductivity is generally very small and depends on the concentration of olive oil added. In the same study, the authors observed a decrease in conductivity of about 2.5% when 10% olive oil was added to an aqueous solution **(Journal of Food Engineering 2006)**.

In summary, although olive oil itself is not conductive, its addition to an ionic aqueous solution can slightly reduce the mobility of the ions and therefore slightly decrease the electrical conductivity of the solution **(Journal of Food Engineering 2006)**.

**NB**: There is no universal standard for the electrical conductivity of canned food, because it varies considerably depending on the type of food and the processing processes used.

### IV.1.3.Determination of BRIX degree

Among the laboratory analyzes carried out on harissa, the measurement of Brix is considered as one of the most decisive to evaluate its quality. Indeed, this parameter makes it possible to highlight the content of soluble dry matter, in other words the percentage of sugars present in the product.

As part of this study, Brix measurements were carried out on 4 separate samples of harissa, at room temperature. The results obtained for each of the samples are summarized in the following table:

**Table 15**: The Brix values of harissa studied.

| Samples | $T_0$ =0 days | $T_1$ =7 days | $T_2$ =15 days | $T_3$ =30 days | standard |
|---|---|---|---|---|---|
| **witness** | 16.5 | 16.4 | 16.3 | 16.2 | |
| **Sample 1** | 16.2 | 17.1 | 16.5 | 16.2 | ≥ 14% |
| **Sample 2** | 15.8 | 16 | 15.8 | 15.9 | |
| **Sample 3** | 16.2 | 16 | 16.2 | 16.0 | |

These data on the levels of soluble dry matter, mainly composed of sugars, provide key information on the composition and the overall quality of the various batches of harissa analyzed.

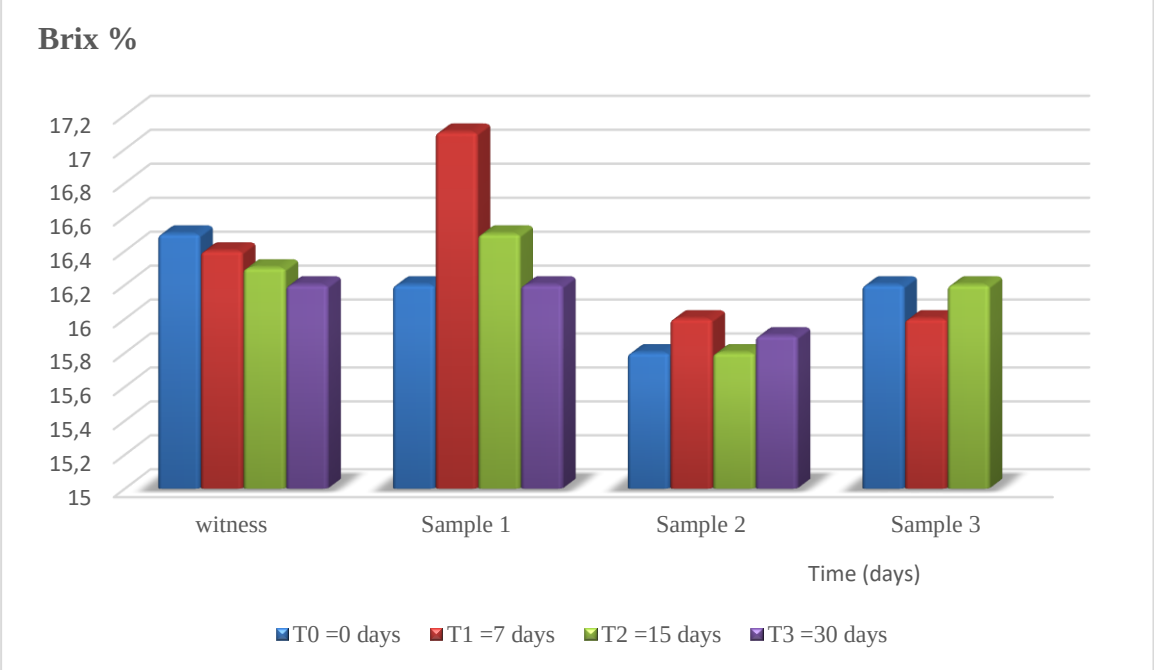



**Figure 53**: Histogram of the Brix of the analyzed samples as a function of time.

According to the results, in the figure 53, the Brix values of the analyzed samples (witness, sample 1, sample 2, sample 3) are between 16.5 and 15.6 from the opening day of the cans up to 30 days. Where we notice a slight decrease in degrees. In both cases, with or without the addition of olive oil.

Olive oil, as a fat, does not directly affect the value of Brix in a food product. The Brix specifically measures the content of soluble sugars or soluble dry matter present. However, the addition of olive oil during the preparation or formulation of a product such as harissa can

have an indirect effect on the Brix in some cases **(Food Analysis), (Principles of Food Chemistry), (Techniques d'analyse et de contrôle dans les industries agroalimentaires)**:

-Dilution of Brix: If olive oil is added in large quantities, it will dilute the concentration of sugars and soluble materials, resulting in a decrease in the Brix value.

-Modification of the solubility: The oil can slightly modify the solubility of the sugars and soluble compounds in the aqueous phase of the product, thus influencing the measured Brix.

-Analytical interferences: The presence of oil can potentially interfere with certain Brix measurement methods, distorting the results if the sample is not properly prepared.

However, in the majority of cases, olive oil has only a small impact on the Brix of a product if it is added in reasonable quantities compared to the other ingredients. The Brix remains mainly influenced by the contents of sugars, soluble carbohydrates and water-soluble compounds present **(Food Analysis), (Principles of Food Chemistry), (Techniques d'analyse et de contrôle dans les industries agroalimentaires)**.

According to our results, the brix values correspond to the standard set at least 14 **(codex R308, 2011)** knowing that this standard is set before the addition of spices.

### IV.1.4.Determination of the titratable acidity

The total content of natural organic acids. Being determined by titration with a strong base (Na OH 0.1 N) by addition of a colored indicator (phenophthalein). The concentration of the acids in the harissa is determined by titrating a test sample with sodium hydroxide until it reaches a pH of 8.1 **[39]**.

The acidity results of the four samples are represented in Table (16) and illustrated by the figure (54).

**Table 16:** The titratable acidity values of harissa studied.

| Samples | $T_0$ =0 days | $T_1$ =7 days | $T_2$ =15 days | $T_3$ =30 days | standard |
|---|---|---|---|---|---|
| **witness** | 3 | 3 | 4 | 4 | |
| **Sample 1** | 3 | 2 | 2 | 2 | ≤ 3.6 |
| **Sample 2** | 3 | 2 | 2 | 2 | |
| **Sample 3** | 3 | 2 | 2 | 2 | |

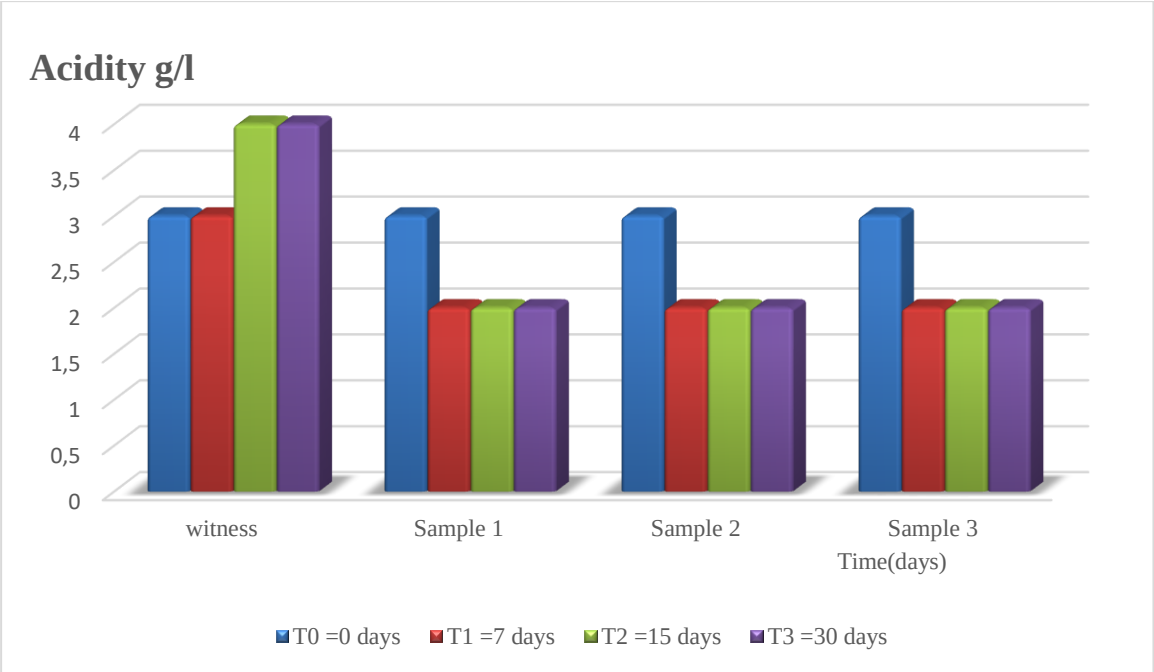



**Figure 54**: Histogram of the titratable acidity of the analyzed samples as a function of time.

According to the results, in the figure 54, the titratable acidity values of the analyzed samples (witness, sample1, sample2, sample3) are between 2 and 4g/l from the opening day of the cans up to 30 days. In (T0) the values of the acidity of the samples are: 3g/l, and consequently the values of the acidity of the samples E1, E2 and E3 are decreased during the conservation period with different doses of olive oil over time except the control: the acidity is increased over time because it is not preserved with olive oil.

The effect of the oil on the acidity can vary depending on the nature of the oil and the production method. However, in general, oil can have a buffering effect on acidity, which means that it can help reduce the acidity of a product. For example, in the case of processed foods, oil can be added as a preservative to reduce acidity and extend the shelf life. This is especially important for products that require specific acidity to guarantee their safety and quality **(Hubert, et *al*)**.

In summary, thanks to its antioxidant, antimicrobial and buffering properties, olive oil rich in phenolic compounds can contribute to reducing the acidity in food by limiting the formation of acids and partially neutralizing the acids present **(Cilla A et *al.*, 2011)**.

According to our results, the acidity values correspond to the standard set at least < 3.6 **(codex alimentarius)**.

### IV.1.5.Determination of ash content

The ash content corresponds to the total amount of mineral residues present in a sample after complete elimination of its organic matter by calcination at very high temperature.

In the case of fruit juices, vegetables or their derived products such as harissa, the ashes represent the mineral salts remaining once all the organic substances (carbohydrates, proteins, lipids, etc.) have been completely burned and the water evaporated.

This measurement of the ash content therefore provides information on the overall content of mineral elements initially contained in the food product. This is an indicator of its richness or depletion in mineral salts.

The residue obtained after calcination thus corresponds to the purely mineral fraction of the analyzed sample, free of any organic component.

The acidity results of the four samples are represented in Table (17) and illustrated by the figure (55).

**Table 17**: The ash content values of harissa studied.

| **Samples** | **$T_0$ =0 days** | **$T_1$ =7 days** | **$T_2$ =15 days** | **$T_3$ =30 days** |
|---|---|---|---|---|
| **witness** | 3.5 | 3.5 | 4 | 3.5 |
| **Sample 1** | 4 | 3 | 4 | 3.5 |
| **Sample 2** | 3.5 | 3 | 3.5 | 3 |
| **Sample 3** | 3.5 | 3.5 | 3 | 2 |

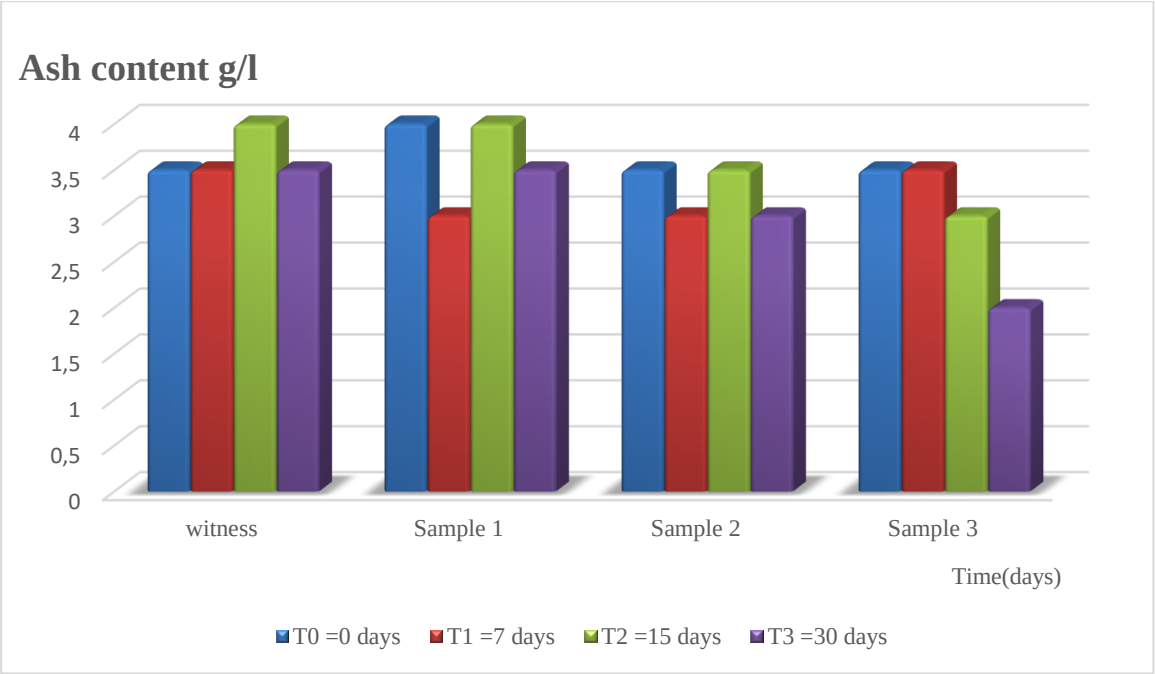



**Figure 55**: Histogram of the ash content of the analyzed samples as a function of time.

According to the results, in the figure 55, the ash content values of the analyzed samples (witness, sample1, sample2, sample3) are between 2 and 4 from the opening day of the cans up to 30 days. We note that all values are constant and close in both cases, with or without the addition of olive oil.

According to research, olive oil would not have a significant effect on the ash content in a food product, since it contains practically no minerals.

In the book **(Jean Graille et *al.*, 1992)**, it is indicated that "refined vegetable oils contain only negligible traces of mineral materials".

Similarly, according to **(S.Suzanne Nielsen, 2010)**, "oils and fats have a very low ash content, less than 0.1% in general".

The standard method of the (**AOAC)** for the determination of ash in oils and fats confirms these low contents, of the order of 0.01 to 0.1%.

The ash content measures the mineral residue remaining after calcination at high temperature of a food sample **(deMan et *al.*, 2018)**. Thus, the addition of olive oil, which is almost completely devoid of minerals, in the formulation of a food product should not significantly impact its final ash content, unless it is added in massive quantities.

In conclusion, the very low mineral content of olive oil makes it possible to consider that its effect on the ash content of food is negligible.

**NB**: There is no universal standard for the ash content of canned food, because it varies considerably depending on the type of food and the processing processes used.

### IV.2. Microbiologic analyzes

Microbiological analyzes are technics for studying microorganisms such as bacteria, yeasts, molds and certain viruses. They make it possible to detect, identify and count these microbes in various media such as food, water, air or clinical samples **(Downes and Ito, 2001)**.

Microbiological analyzes are essential to ensure the safety and quality of canned food. Despite the heat treatment applied during production, microbial contamination can occur due to process defects or the manufacturing environment **(Njoku et *al*., 2014)**.

The main objective is to evaluate the microbiological quality, safety and compliance of products with current standards **(Madigan et *al*., 2018),** and also is to detect and count pathogenic or altered microorganisms likely to develop, representing a health risk or compromising the quality of the product **(Mossel et *al*., 2003)**.

Our microbiological analysis was examined for 1 month at 4 different samples

#### IV.2.1. Effect of olive oil and time on the development of microorganisms

Olive oil has antimicrobial properties that allow it to inhibit the development of certain undesirable microorganisms in food. However, the effectiveness of its inhibitory effect depends on the type of targeted microorganism as well as the shelf life of the food product. The compounds present in olive oil, in particular phenolic compounds such as oleuropein and hydroxyl-tyrosol, are responsible for this antimicrobial activity. Their action varies depending on the microorganism considered, whether it is pathogenic bacteria, molds or alteration yeasts. In addition, the inhibitory effect of olive oil may subside over the storage time.

#### IV.2.2. Microbiological analysis results

1. **Results of Research of total mesophilic aerobic germs (FTAM)**

The table below expresses the number of total aerobic mesophilic germs on 4 samples taken at different times and different doses of olive oil.

**Table 18:** Numbers of the counted germs of total mesophilic aerobic germs.

| Samples | $T_0$ =0 days | $T_1$ =7 days | $T_2$ =15 days | $T_3$ =30 days |
|---|---|---|---|---|
| **witness** | 30 **UFC /g** | 60 **UFC /g** | 100 **UFC /g** | 310 **UFC /g** |
| **Sample 1** | 30 **UFC /g** | 25 **UFC /g** | 15 **UFC /g** | Absence |
| **Sample 2** | 25 **UFC /g** | 20 **UFC /g** | Absence | Absence |
| **Sample 3** | 30 **UFC /g** | 22 **UFC /g** | 18 **UFC /g** | Absence |

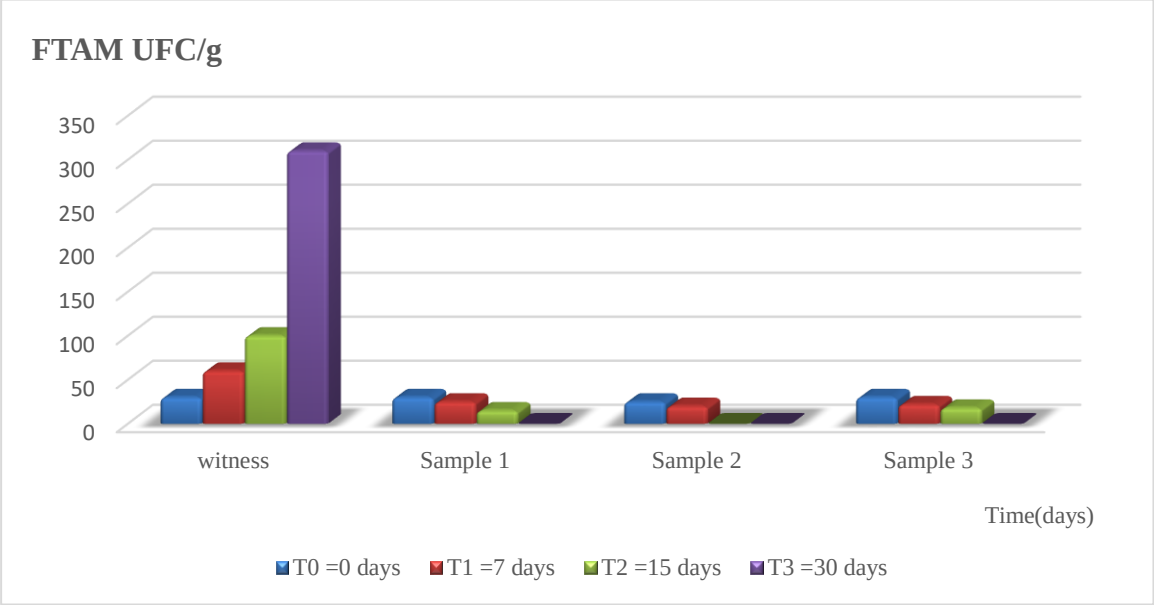



**Figure 56**: Graphical presentation of the results of the FTAM enumeration.

After counting these germs, we found 30 UFC/g in the first day of opening of the 4 cans of harissa T0, where the witness had an increase in the microbial load during the storage period with a maximum value of 310 UFC/g at T3.While the rest of the cans (sample 1,2,3) contain different amounts of olive oil, we notice that there is a decrease in the microbial load during the conservation period with a maximum value of 30 UFC/g. Which over time has gradually decreased until it becomes non-existent.

The increased microbial charge of the witness is explained by the presence of growth-promoting factors such as storage conditions (long duration) that favor the reproduction of organisms in food **(Snyder and Sugie, 1986)**.

The reason for the decrease in the microbial charge of the samples 1, 2 and 3 due to the Olive oil which has antimicrobial properties thanks to its composition of phenolic compounds, in particular oleic acid derivatives such as oleuropein, tyrosol and hydroxytyrosol. These

compounds can inhibit the growth of total mesophilic aerobic germs in food products.have shown that extra virgin olive oil significantly reduces the growth of total aerobic mesophilic bacteria **(Medina et *al.*, 2006)**.

The inhibitory effect of the oil is observed very well olive oil on microbial development. We notice that there is a linear relationship between the volume of the oil added and the rate of germs obtained. Therefore, olive oil has an inhibitory effect on microbial growth that can be closely linked to the presence in virgin olive oil of substances with this inhibitory power. **(BOUHADID et *al.*, 2022)**.

Despite this, the results are lower than Algerian standards **(Jora, 2017)** which shows that the number of mesophilic aerobic flora is $10^5$.

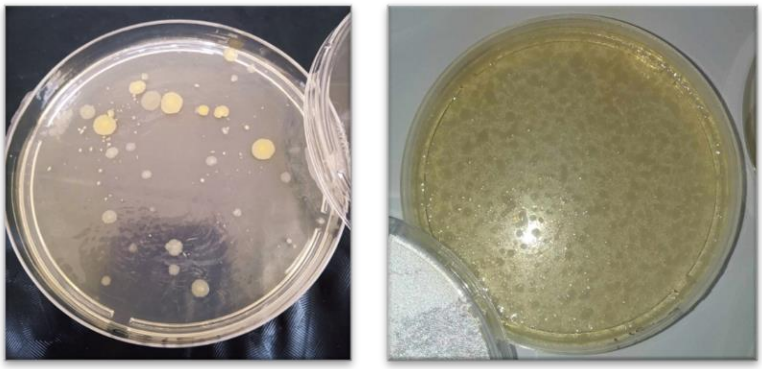


**Figure 57**: Representing the search for FTAM on PCA **(Personal photo 2024)**.

**2. Results of Research and enumeration of yeasts and molds**

Some yeast and mold spores are resistant to extreme conditions such as heat, freezing and antibiotics. Thus, strict quality control of food products is essential to prevent their proliferation and guarantee food safety **(Maude, 2019)**.

The table below expresses the number of total aerobic mesophilic germs on four samples taken at different times and different doses of olive oil.

**Table 19:** Numbers of the counted germs of yeasts and molds.

| **Samples** | **$T_0$ =0 days** | **$T_1$ =7 days** | **$T_2$ =15 days** | **$T_3$ =30 days** |
|---|---|---|---|---|
| **witness** | 16 **UFC /g** | 20 **UFC /g** | 45 **UFC /g** | 50 **UFC /g** |
| **Sample 1** | 17 **UFC /g** | Absence | Absence | Absence |
| **Sample 2** | 16 **UFC /g** | Absence | Absence | Absence |
| **Sample 3** | 18 **UFC /g** | Absence | Absence | Absence |

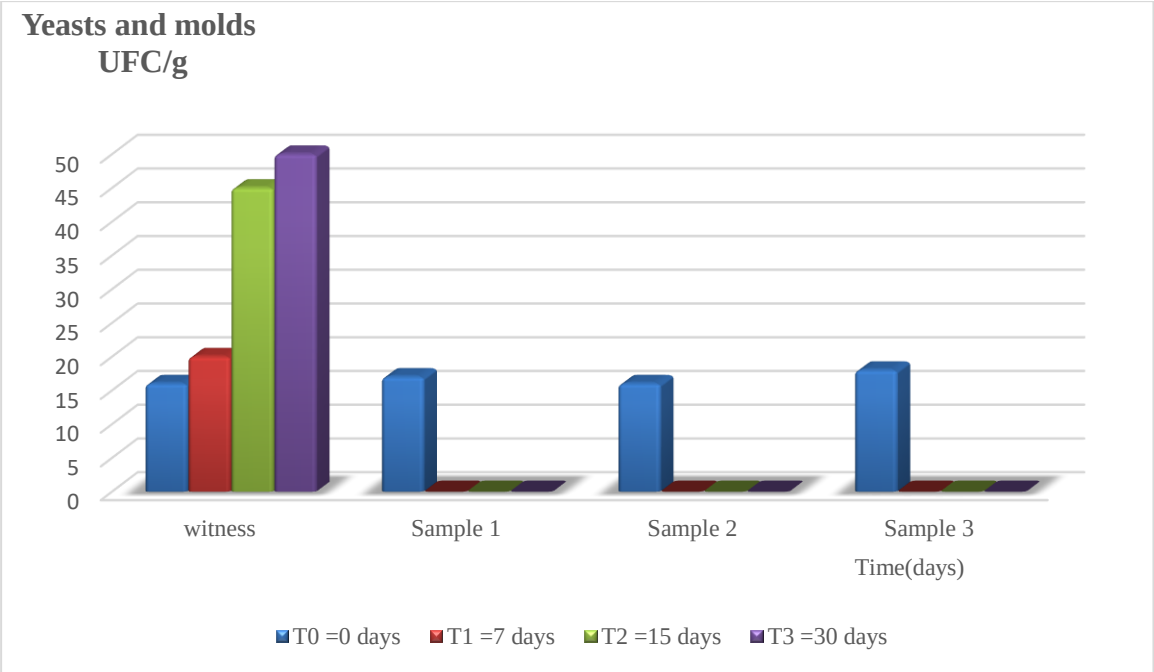



**Figure 58**: Graphical presentation of the results of the yeasts and molds.

According to the graph examination illustrated in Figure (58) and which presents the evolution of yeasts and molds in the 4 harissa samples during their conservation after cans opening, the analysis of these samples revealed various results in terms of microbial load.

After counting these germs, we found (16 UFC/g) in the first day of opening of the 4 harissa cans, T0 where the witness had an increase in the microbial load during the storage period with a maximum value of 50 UFC/g at T3.While the rest of the cans (sample 1,2 and 3) contain different amounts of olive oil, we notice that there is a decrease in the microbial load during the conservation period with a maximum value of 18 CFU/g. Which over time has gradually decreased until it becomes non-existent.

The increased microbial charge of the witness is explained by the growth of fungi in the harissa because its contains oils, sugars and proteins from peppers, garlic, spices, which can serve as nutritional sources for mushrooms **(Deak, 2008)**. Molds usually need a higher water activity (Aw) than bacteria to grow. According to a study by **(Pitt and Hocking, 2009),** most food spoilage molds cannot develop below an Aw of 0.80.

The reason for the decrease in the microbial charge of the samples 1,2 and 3 due to the Olive oil which is rich in phenolic compounds can also inhibit the growth of certain molds and yeasts responsible for food spoilage **(Gourama and Bullerman, 1987; Tafakori et *al.*, 2015)**.

It should be noted that these results comply with the standards (**NF ISO 7954/88)**, the number of which must be less than $10^3$ which set acceptable limits for the microbial presence in food products.

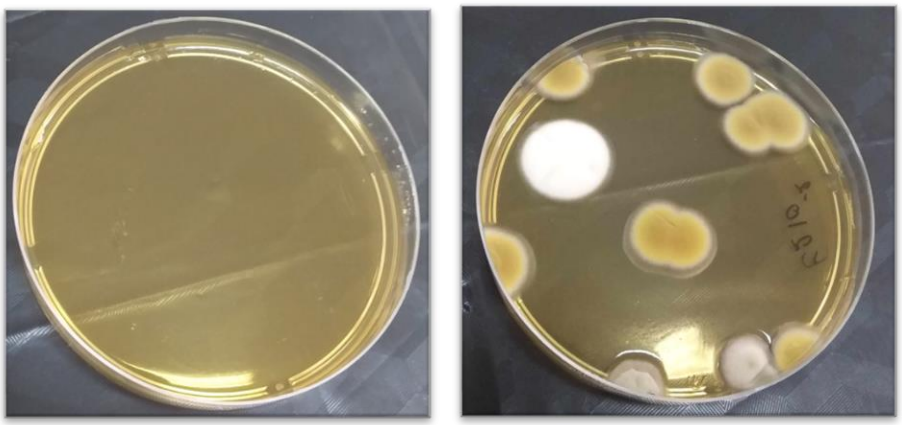


**Figure 59**: Representing the search for the yeasts and molds on OGA **(Personal photo 2024)**.

**3. Results of enumeration of total coliforms and fecal coliforms**

The analysis of total and fecal coliforms is an essential tool to assess the microbiological quality of harissa and detect possible contamination. Coliforms are indicator bacteria whose presence usually signals insufficient hygienic conditions during the production, processing or handling of food.

The enumeration of total coliforms provides a general indication of the level of hygiene, while the search for fecal coliforms, such as Escherichia coli, makes it possible to specifically detect a contamination of fecal origin, potentially linked to the presence of enteric pathogens.

The results of these microbiological analyses on the harissa allow the competent authorities to assess the potential health risks and to take appropriate measures. This may involve recalling contaminated batches, inspecting production facilities or setting up training courses to reinforce good hygiene practices.

This microbiological surveillance thus contributes to guaranteeing the safety of the harissa and to protecting the health of consumers against the risks of food toxi-infections.

**3.1. Total coliforms**

Concerning the results of the enumeration of total coliforms are expressed in the table below:

**Table 20**: Numbers of the counted germs of Total coliforms.

| Samples | $T_0$ =0 days | $T_1$ =7 days | $T_2$ =15 days | $T_3$ =30 days |
|---|---|---|---|---|
| **witness** | Absence | Absence | 45 **UFC /g** | 75 **UFC /g** |
| **Sample 1** | 30 **UFC /g** | 15 **UFC /g** | Absence | Absence |
| **Sample 2** | 20 **UFC /g** | Absence | Absence | Absence |
| **Sample 3** | 18 **UFC /g** | Absence | Absence | Absence |

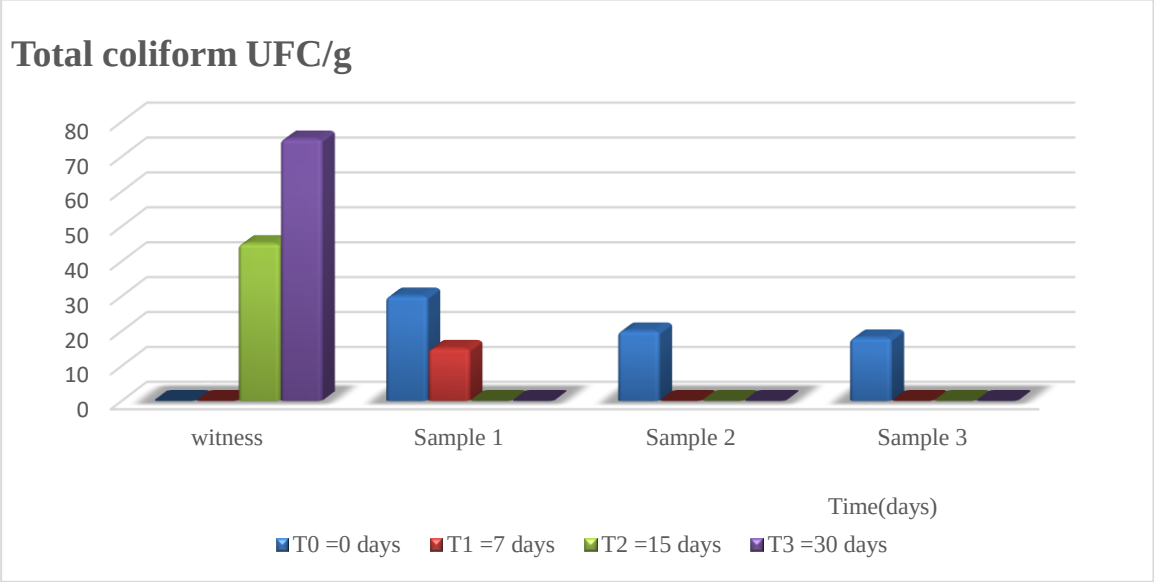



**Figure 60**: Graphical presentation of the results of the Total coliforms.

According to these results obtained, the total absence of total coliforms for the control was noted in T0 (after opening), but in T2 (after 15 days), T3 (after 30 days) they begin to increase when it reaches (75 UFC/g).and for the scallops E1,E2,E3, the presence of total coliforms is observed in T0 (after opening) (30 UFC/g) and during preservation with olive oil In different quantities, a gradual disappearance is observed until there is no more in T3.

These results of the absence in the witness (T0, T1) may be due to pasteurization, where no microorganisms have been detected/ found, which highlights the effectiveness of the heat treatment in the elimination of pathogenic microorganisms and the reduction of vegetative flora in the product. This result demonstrates the importance of pasteurization as a safety measure food to guarantee the quality and safety of the products. By eliminating unwanted microorganisms, pasteurization contributes to prolonging the shelf life of food while ensuring their safety for consumers **(Olivier, 2011)**.

On the other hand, we explain the appearance of total coliforms in witness (T0, T1) by:

1-Contaminated raw materials (spices, vegetables, oil, etc.): If the spices, vegetables or other ingredients used to prepare the harissa are already contaminated with coliforms, this may affect the finished product **(Hampikyan and Ugur, 2007)** detected coliforms in 30% of the spice samples analyzed in Turkey.

2-Inadequate hygienic conditions during production: **(The FAO/OMS Cod., 2009)** emphasizes the importance of compliance with good hygiene practices during the production of spices to prevent contamination, a lack of hygiene of equipment, work surfaces, water used or poor staff hygiene can introduce coliforms into the harissa.

3-Cross-contamination: Contact with surfaces, utensils or other food contaminated with coliforms can lead to their transfer into the harissa **(Vij et *al*., 2006),** have shown that poorly cleaned work surfaces and utensils are potential sources of coliform contamination in food.

4-Abusive times/Temperatures during production/storage According to **(Health Canad., 2012),** a proliferation of coliforms can occur if the food is exposed to temperatures favorable for their growth, If the harissa is not maintained at appropriate temperatures during production, transport or storage, the multiplication of coliforms can take place.

5-Use of contaminated water: Infiltration of contaminated water: The use of unsafe water for preparation or rinsing can be a source of contamination by coliforms The **( FAO/OMS., 2009)** recommends the use of drinking or treated water to avoid the introduction of microbial contaminants.

6-Defective packaging: Defective packaging: Defective or non-hermetic packaging may allow the introduction of coliforms into the finished product **(Pal et *al*., 2017)** observed an increase in the microbial load, including coliforms, in poorly packaged spices.

Strict adherence to good hygiene practices at all stages, the quality of raw materials and appropriate storage conditions are essential to prevent coliform contamination in harissa.

The reason for the decrease in the microbial charge of the samples 1,2 and 3 due to:

Several studies have demonstrated that olive oil, especially extra virgin olive oil, can help reduce the levels of total coliforms in various foods thanks to its natural antimicrobial properties. This effect is mainly attributed to the phenolic compounds present in olive oil, such as hydroxytyrosol, tyrosol and oleuropein.

In a study on harissa, a typical condiment **(Challouf et *al*., 2011)** found that the addition of olive oil significantly reduced total coliform levels during storage.

Despite this, the results are lower than Algerian standards **(Jora., 2017)** which shows that the number of Total coliforms flora is $<10^2$.

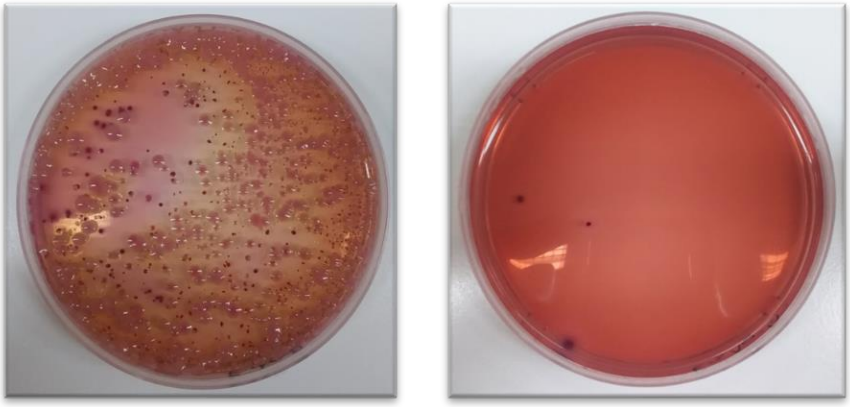


**Figure 61**: Representing the search for the Total coliforms on VRBL **(Personal photo 2024)**.

### 3.2. Fecal coliforms

Concerning the results of the enumeration of fecal coliforms are expressed in the table below:

**Table 21**: Numbers of the counted germs of fecal coliforms.

| Samples | $T_0$ =0 days | $T_1$ =7 days | $T_2$ =15 days | $T_3$ =30 days |
|---|---|---|---|---|
| **witness** | Absence | Absence | Absence | Absence |
| **Sample 1** | Absence | Absence | Absence | Absence |
| **Sample 2** | Absence | Absence | Absence | Absence |
| **Sample 3** | Absence | Absence | Absence | Absence |

Our results reveal a total absence of fecal coliforms from the opening of the box until the end of our study.

Algerian standards require a total absence of fecal coliform **(JORA., 2017)**. So according to these standards all the samples are in good quality.

### 4. Search results for fecal streptococci

Concerning the results of the enumeration of fecal streptococci are expressed in the table below:

**Table 22**: Numbers of the counted germs of Fecal streptococci.

| Samples | $T_0$ =0 days | $T_1$ =7 days | $T_2$ =15 days | $T_3$ =30 days |
|---|---|---|---|---|
| **witness** | Absence | Absence | Absence | Absence |
| **Sample 1** | Absence | Absence | Absence | Absence |
| **Sample 2** | Absence | Absence | Absence | Absence |
| **Sample 3** | Absence | Absence | Absence | Absence |

Our results reveal a total absence of fecal streptococci from the opening of the cans until the end of our study.

Algerian standards require a total absence of fecal streptococci **(Abdaoui et *al*., 2016)**. So according to these standards all the samples are in good quality.

**5. Results of sulfito-reducing anaerobic bacteria (ASR)**

Concerning the results of the enumeration of the sulphito-reducing are expressed in the table below:

**Table 23**: The enumeration of the sulphito-reducing Clostridium

| Samples | $T_0$ =0 days | $T_1$ =7 days | $T_2$ =15 days | $T_3$ =30 days |
|---|---|---|---|---|
| **witness** | Absence | Absence | Absence | Absence |
| **Sample 1** | Absence | Absence | Absence | Absence |
| **Sample 2** | Absence | Absence | Absence | Absence |
| **Sample 3** | Absence | Absence | Absence | Absence |

Our results reveal a total absence of sulphito-reducing Clostridium from the opening of the box until the end of our study.

Algerian standards require a total absence of fecal coliforms **(NF ISO 7937/05)**. So according to these standards all the samples are in good quality.

The total absence of sulfito-reducing Clostridium is an essential criterion to guarantee the safety of food, in particular canned goods and stable products, in accordance with current microbiological standards **(NRCP, 2010; JORA, 2019).**

## 6. Results of pathogenic germs

### 6.1. Salmonella

Concerning the results of the enumeration of Salmonella are expressed in the table below:

**Table 24:** The enumeration of Salmonella.

| Samples | $T_0$ =0 days | $T_1$ =7 days | $T_2$ =15 days | $T_3$ =30 days |
|---|---|---|---|---|
| **witness** | Absence | Absence | Absence | Absence |
| **Sample 1** | Absence | Absence | Absence | Absence |
| **Sample 2** | Absence | Absence | Absence | Absence |
| **Sample 3** | Absence | Absence | Absence | Absence |

The results obtained indicate a total absence of salmonella in all samples of harissa during the study period.

These results correspond to the **(JORA., 2017)** standard which indicates the absence of salmonella in canned food.

The total absence of sulfito-reducing Clostridium is an essential criterion to guarantee the safety of food, in particular canned goods and stable products, in accordance with current microbiological standards **(NRCP, 2010; JORA, 2019).**

### 6.2. Staphylococci

Concerning the results of the enumeration of Staphylococci are expressed in the table below:

**Table 25:** The enumeration of Staphylococci.

| Samples | $T_0$ =0 days | $T_1$ =7 days | $T_2$ =15 days | $T_3$ =30 days |
|---|---|---|---|---|
| **witness** | Absence | Absence | Absence | 2 **colony** |
| **Sample 1** | Absence | Absence | Absence | Absence |
| **Sample 2** | Absence | Absence | Absence | Absence |
| **Sample 3** | Absence | Absence | Absence | Absence |

According to the results obtained and presented in the figure 60, a total absence is observed of the microbial load of *Staphylococcus aureus* in all samples from harissa (witness, Sample 1, 2 and 3) and this absence has not increased during conservation, since the first day up to T3

(After 30 days), these results correspond to the **(Codex A)** standard which indicates the absence of Staphylococci in canned food.

However, in T3 a slight increase for the control of 2 colonies was noted, it can be explained by external contamination, because Staphylococcus can be introduced into the jam from an external source such as air, hands or other surface contaminated during the preparation of the harissa but more probably because of the wrong handling during the analysis. The risk of contamination of a food when professionals in the food sector do not wash themselves properly before touching the food **(Jonathan., 2021).**

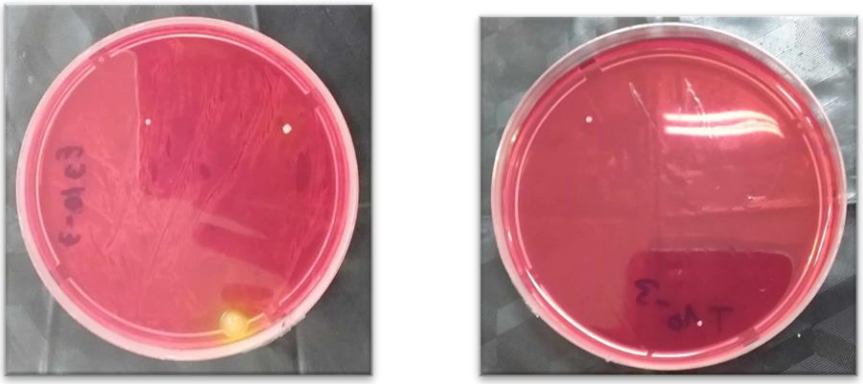


**Figure 62**: Representing the search for the Staphylococci on chapman **(Personal photo 2024)**.

### 6.3. Pseudomonas and shigella

Our results reveal a total absence of pseudomonas and shigella from the opening of the cans until the end of our study.

Algerian standards require a total absence of pseudomonas and shigella **(JORA., 2017)**, So according to these standards all the samples are in good quality.

After identification by API 20E on SS and Mac Conkey media, the results indicate the absence of salmonella and Shigella and the presence of *Paenibacillus macerans* .

*Paenibacillus macerans* is a sporulating, mesophilic bacterium, generally isolated from the environment (soil, water) and food products. It can cause the alteration of certain foods **(Grönlund et *al*., 2011)**.

*P. macerans* is not generally considered pathogenic for humans, but some strains can cause opportunistic infections in immunocompromised people **(Logan, 2012)**.

After identification by API 20E on VRBL media, the results indicate the presence of ***Bacillus licheniformis*.**

*B. licheniformis* is a saprophytic telluric germ (soil and water) widely distributed in nature. It is capable of sporulating which gives it a great longevity, as well as resistance to extreme temperatures, desiccation and chemicals **(Niall et *al*., 2000).**

The pathogenicity of this bacterium seems to be linked to the production of an extracellular polymer of glutamic acid which explains the characteristic mucous appearance of the colonies **(Soler et *al*, 2004).**

After identification by API 20NE on VRBL media, the results indicate the presence of ***Pseudomonas luteola.***

P. luteola is a rod-shaped bacterium, mobile thanks to a polar flagellum. It is catalase positive and oxidase positive. This species produces a yellow-orange pigment called xanthine **(Stanier et al., 1966; Oyaizu and Komagata, 1983).**

*P. luteola* is generally not considered pathogenic for healthy humans. However, it can cause opportunistic infections in immunocompromised patients, including pneumonia and bacteremia **(Moore et al., 2006; LiPuma and Currie, 2010).**

After identification by API Staph on the chapman medium, the results indicate the presence of

***Staphylococcus xylosus.***

*Staphylococcus xylosus* are indicators of human (or animal or original) contamination since these germs are naturally present on the skin and mucous membranes of humans and animals; therefore, the absence in our tomatoes does not pose the risk of enterotoxicosis **(Cuq, 2007).**

In addition, this species is found at the level of surfaces and soils, and in agri-food environments **(Corbiere, 2006)**. *S. xylosus* is also described as the majority species of combined fodder where it is found in each of the elements that compose it, grains and green cereal plants, but also in soil and dust samples from the manufacturing sites **(Pioch et *al*, 1988).**

These results comply with the standards described by the Canadian Standards Council (N° 131), which requires a number not exceeding 104 for *Staphylococcus xylosus*.

**7. Result of biochemical tests (API gallery, catalase test, oxidase test)**

**Table 26**: Result of the biochemical tests (API gallery, catalse test, oxidase test).

| **Culture media** | **VRBL** | **Mac Conkey** | **SS** | **Chapman** | **VRBL** |
|---|---|---|---|---|---|
| **The germ search** | **Total Coliform** | **Shigelles** | **Salmonelles** | **Staphylococci** | **Total Coliform** |
| **Catalase Test** | + | + | + | + | + |
| **Oxydase Test** | - | - | - | - | + |
| **Identified germ by The Api Gallery** | *Bacillus licheniformis* ***(Api 20E)*** | *Paenibacillus macerans* ***(Api 20E)*** | *Paenibacillus macerans* ***(Api 20E)*** | *Staphylococcus xylosus.* **(Api Staph)** | *Pseudomonas luteola.* ***(Api 20NE)*** |

**Figure 63**: Representing the results of the API galleries **(Personal photo 2024)**.

# Conclusion

It is essential to take measures to ensure the quality of the food products stored from the beginning of the production chain and to intervene on the production sites in order to eliminate all products presenting potential risks to the health or safety of consumers.

Our job is to evaluate the microbiological and physicochemical quality of a food product according to the shelf life (case of Harissa).

After the physico-chemical study of the harissa, all the results of the analyzes showed that :

- the pH of the samples has remained acidic overall since the first day the cans were opened. On the other hand, we have observed that the storage time of canned harissa before and even after opening the cans does not alter the Brix in any way which has remained within the required standard (≥ 14% ). The conductivity results and the ash content are at the required levels and remain stable during the storage period.the titratable acidity revealed values in accordance with the standards ( ≤3.6).

These results testify that the harissa is of satisfactory physico-chemical quality.

- The results of the microbiological analyses carried out on the 4 harissa samples with olive oil demonstrate their perfect compliance with the standards in force. Indeed, the counts of total mesophilic aerobic flora (FTAM), total coliforms (CT) and faecal (CF), faecal streptococci, as well as yeasts and molds are below the established regulatory thresholds.
- In addition, no pathogenic germs such as sulfito-reducing Clostridium, Salmonella, Shigella, Pseudomonas or Vibrio have been detected. Only a few Staphylococcus xylosus have been counted at a very low level, below $10^4$ UFC/g, not presenting a major health risk.

In view of the results of our study, we can conclude that the use of olive oil as a preservative for harissa effectively contributes to a better preservation of its quality over a period of (30 days). Olive oil, due to its antioxidant properties and its stability against heat treatments, has proven to be an excellent natural preservative for this condiment.

# Bibliographic References

## Bibliographic references


### A

- **Abdaoui, A., Necaibia, N., Saadi, S.,( 2016)**. Suivi de Qualité de Confiture d'abricot et Application de la Méthode HACCP : Cas de la société Amor BenAmor (Nord-est Algérien).
- **Abdellioui S., Boukhdim A., Hamzaoui H.,(2012).** Qualité microbiologique d'un écosystéme lotique cas de l'oued El Kebir Ouest (Skikda,Nord-Est algérien). Mémoire de master. Université 8 Mai 1945 Guelma, 123p.
- **AFNOR V76 - 124,( 1994)** .cited by AFTER, 2011**.**
- **AFNOR, (1974).** (Association Française de Normalisation). Détermination de l'acidité Titrable.
- **Afssa,( 2009).** Risques liés à la présence de moisissures et levures dans les eaux conditionnées, agence française de sécurité sanitaire des aliments, 54.
- **Amira W, (2008**). Degré de contamination de l'eau de la mare Redjla (Taher) par les nitrates : Détermination de la qualité physicochimique et microbiologique de l'eau. Mémoire de magister. Université de Jijel. 103 p. Auclair G, 2009. Lignes directrices et normes pour l'interprétation des résultats analytiques en microbiologie Direction Générale De L'alimentation (Le. Conseil Canadien Des Normes).
- **AOAC** (Association of Official Analytical Chemi).
- **Apab,(Décembre 2011).** Guide des bonnes pratiques d'hygiène, filière algérienne des jus de fruits, nectars et produits dérivés. Association algérienne des producteurs de boissons.
- **Azerou H., Amaouz H., (2020).**Etude des caractéristiques physicochimiques, nutritionnelles et anti oxydantes du piment (Capsicumspp).Mémoire de master.Université A-Mira Bejaia. 64p.

### B

- **Bara Ya,( 2016 ).** Étude comparative de la qualité physicochimique et bactériologique de l'eau du barrage de Hammam Debagh avant et après traitement Cas de la station de traitement de Hammam Debagh – Guelma. Mémoire de master, université de 08 Mai 1945 Guelma, Algérie. 89p.
- **Benabid H. (2009).** Caractérisation de l'huile d'Olive Algérienne, Apports des méthodes chimiométriques. INATAA. Université Mentouri De Constantine, Algérie.

- **Bennacer L, (2018**).« production et transformation de la tomate industrielle dans le bassin de Guelma une filière en développement ».sciences et technologie ; n°47, juin 2018, pp72, 79p.
- **Berche.,Gaillard J.L .,et Simouet M, (1988).** Bactériologie, Les Bactéries Des Infections Humaines. Flammarion Médecine Sciences. 660p.actériologie médicale et vétérinaire. Systématique bactérienne. Doin, France. 371p
- **Bitton G, (1999): Waste** water microbiology. 2éme éd. John Wiley & Sons. NY. 578p
- **Bordjiba Kaouther.. Douakha Djaza.(2023)**: Suivi de la qualité de la harissa, de l'eau brute et contribution à la mise en place du système HACCP au niveau de la conserverie CAB. Mémoire de master, université de 08 Mai 1945 Guelma, Algérie.92p.
- **Boskou, D., Blekas, G.,and Tsimidou, M. (2006).**Olive oil composition. In Olive Oil: Chemistry and Technology (pp. 41-72). AOCS Press.
- **BoudjirI and Zehar S, (2019).** Evaluation de la qualité physico-chimique et microbiologique du lait de brebis. Mémoire de Master. Université Mohamed El Bachir El Ibrahimi-Bordj Bou Arreridj. P33.
- **Bougeois C. M. et Leveau J,(1996).** Teclmique d'analyse et de contrôle dans les industries agroalimentaires. Paris: Lavoisier TEC et DOC, 1996, 331p.
- **Boughida Mayssa., Merabet Fatma Zahra., Nemouchi Meriem.(2023):**Etude de la qualité microbiologique de la conserve du concentré de tomate. Mémoire de master, université de 08 Mai 1945 Guelma, Algérie.61p.
- **Boukhatem.,(2013) :Boukhatem L., 2013**: Etude de la sensibilité aux antibiotiques des bacilles à Gram négatif non fermentants isolés au niveau du service de réanimation du CHU de Tlemcen. Mémoire de Master. Université Aboubaker Belkaid, Tlemcen, Algérie. 131p.
- **Boussaid F., HarrazF ,(2019):** Analyse et évaluation de la qualité physicochimique des conserves et des eaux.mémoire de master. Université 8 Mai 1945, Guelma.70p

**C**

- **Camille Delarras (2007).** Microbiologie pratique pour le laboratoire d'analyse ou de contrôle sanitaire. Lavoisier. Editions TEC and DOC. 11, rue la voisier F-75008 Paris. 129p.
- **Carbonnelle D., Kouyoumdjian S. et Audurier A. (1988)**. Bactériologie médicale techniques usuelles. Méd. Mal. Inf. Article.France. 251p.
- **Carrillo, BA, McFarlin, R., Pane, PP et Moreno, FAR (2022).**Un nouveau système d'assainissement pour prévenir la croissance microbienne dans les aliments : un examen complet. Frontières en microbiologie, 13, 2135-2158.
- **CEAEQ, (2006).** Centre d'Expertise en Analyse Environnementale du Québec: Recherche et dénombrement des entérocoques: méthode par filtration sur membrane. MA. 700-Ent 1.0,

Rév. 3. Ministère du Développement durable, de l'Environnement et des Parcs du Québec. 23p.

- **Challouf, (2011)** .J. Food Process. Preserv. 35(4):512-520
- **Cilla, A.(2011).** Food Research International, 44(5), 1314-1319.
- **Clausen E.M., Green B.L. and Litsky W. (1977)**. Fecal streptococci: indicators of pollution. Am. Soc. Test. Mat. (ASTM). 635: 247-264.
- **Codex Alimentarius (1981).** Norme codex pour les huiles d'olive et les huiles de grignons d'olive. Codex Stan 33-1981.
- **Codex Alimentarius (2021).** Norme codex pour les huiles d'olive et les huiles de grignons d'olive. Codex Stan 33-1981.
- **Codex Alimentarius**. Normes alimentaires internationales.
- **CODEX STAN 192-1995 (2015).** Codex Alimentarius. Norme générale pour les additifs alimentaires.
- **Codex R308 (2011).** Codex Alimentarius des normes alimentaires internationales.
- **Corbiere Morot-Bizot S, Leroy S, and Talon R (2006).** Staphylococcal community of a small unit manufacturing traditional dry fermented sausages. International Journal of Food Microbiology. 108, 210-217
- **Cuq J.L (2007).** Microbiologie Alimentaire. Université Montpellier ri (Département de Sciences et Technologies des Industries Alimentaires). Poly Tech Montpellier.

**D**

- **Daoussi, R., Scognamiglio, G., Mastrobuoni, F., (2022).** Innovative strategies to improve the quality and safety of traditional harissa. Foods, 11(14), 2186.
- **Deak, T. (2008).** Handbook of Food Spoilage Yeasts. CRC Press.
- **Delarras C. (2003).** Surveillance sanitaire et microbiologique des eaux. Édition Lavoisier
- **Delarras C. (2014).** Pratique en microbiologie de laboratoire Recherche de bactéries et de levuresmoisissures. Tec & Doc/Lavoisier. 800 p. p.432.p. p.443.p.435. p.(257-258). p.280. p(237). p(257).p. (595-598).p.624. p572.p. page340-341.p. p.113.p.103
- **Dhouioui, M., Moro, S., Budryn, G., (2022).** Aromatic profiles and sensory descriptors of two types of North African harissa: characterization through GC-MS and SPME-GC/MS. Foods, 11(17), 2682.
- **Doublier, J.-L. T.-F. (2009).** Agents épaississants et gélifiants de nature glucidique. Additifs et auxiliaires de fabrication dans les industries agroalimentaires (4e ed.).

• **Downes and Ito (2001).** Méthodes d'examen microbiologique des aliments et de l'eau. Springer.

**F**

- **Farrow J.A.E., Kruze J., Phillips B.A., Bramley A.J. and Collins M.D. (1984**): Taxonomic studies of S. bovis and S. equinus: description of S. alactolyticus sp. no. and S. saccharolyticus sp. nov. System. Appl. Microbiol. 5:467-482.
- **Fondio L.(2009)** .Bien cultiver le piment Ed, centre technique national de recherche agronomique, Côte d'Ivoire. p30.
- **"Food Analysis"** par S. Suzanne Nielsen (5ème édition, Springer)
- **Foughali (2021).** Contribution aux suivis de la qualité du concentré de tomate de la conserverie ZIMBA et des eaux de chaudières.

**G**

- **Ghedira K. (2008).** L'olivier. Journal de la phytothérapie, vol. 6, pp. 83-89 .
- **Ghanbari, R., Zarei, M., Ebrahimpour, A., Abdul-Hamid, A., Ismail, A., and Saari, N. (2019).** Olive oil quality prediction using dielectric spectroscopy and chemometrics. Food Analytical Methods, 12(2), 373-383.
- **Giuffrè AM. (2013).** Influence of harvest year and cultivar on wax composition of olive oils. European Journal of Lipid Science and Technology, 115(5), 549-555.
- **Gourama, H., and Bullerman, L. B. (1987).** Journal of Food Protection, 50(6), 489-495.
- **Grasselly. D (2000).** Pour un produit de qualité. Edition centre technique interprofessionnel des fruits et légumes.
- **Grönlund, (2011).** Int J Syst Evol Microbiol, 61(8), 1744-1748.
- **Guiraud J.P. (1998).** Microbiologie alimentaire. Dunod. Paris, France. 651 p.

**H**

- **Hadji F, (2020).** Analyse physico-chimique et microbiologique de l'eau. Mémoire de Master, Université 08 mai 1945 Guelma.
- **Hampikyan, H.,and è Ugur, M. (2007).** The microbiological quality of retail food samples in Turkey. Internet Journal of Food Safety, 9, 67-76.
- **Hocine F,( 2022à :** Contribution à l'étude de la qualité microbiologique de l'eau de quelques sources naturelles dans la région de Guelma. Mémoire de master. Université 08 mai 1945 Guelma, 119p.

- **Hubert DOMONHEDO, David CROS, Léifi NODICHAO, Norbert BILLOTTE et Corneille AHANHANZO .**Enjeux et amélioration de la réduction de l'acidité dans les fruits mûrs du palmier à huile, Elaeis guineensis, Jacq.

**I**

- **Isabelle T,( 2002à.** Filières laitières en Afrique et points critiques pour la maîtrise des laits et produits laitiers, diplôme d'études supérieures, Université Montpellier, p7.
- **ISO 4833,( 2003).** Microbiologie des aliments-Méthode horizontale pour le dénombrement des micro-organismes-Technique par comptage des colonies.

**J**

- **Jean Graille, Tec and Doc Lavoisier, (1992 ).** Analyses of fats.
- **Joffin and Leyrol, (2001).** Microbiologie Technique 1 : dictionnaire des techniques. 3 IIème éditions. CRDP d'Aquitaine. p320.
- **JORA, (2017).** Journal officiel de la republique algerienne democratique et populaire.
- **JORA (2019).** Norme algérienne NA13 - Denrées alimentaires et conditions générales d'hygiène. Journal Officiel de la République Algérienne.
- **Journal of Applied Chemistry** (Vol. 3, No. 2, 2013).

**K**

- **Kari N., Laifaoui S., (2013).** Etude de la résistance aux Antibiotiques Chez les Bacilles à Gram négatif isolés à partir des Effluents de deux Hôpitaux de la Wilaya de Béjaia (Akbou et Sidi Aich). Mémoire de Master. Université Abderrahmane MIRA. 95p
- **Khineche E., (2022).** Situation de la conservation des légumes (la tomate, l'oignon et le piment) à Froid dans la région de _BISKRA_.mémoire de master . Université Mohamed Khider , BISKRA.53p**.**
- **-( K. Horri et *al.*,2015) : K. Horri, A. Dahane, M. Maatoug ,(2015).**Problématique du développement des industries agroalimentaires en Algérie. Eur. Sc. J. 2015
- **Kothari S., Joshi A., Kashawa S., etc.( 2010).**Piment fort-une revue sur la culture et lacomposition des tissus.Progrès de la biotechnologie.28:35_48.
- **Krishna de L, (2004).** Capsicum: Le genre Capsicum. Plantes Médicinales et Aromatiques-Profils industriels. Presses du CRC, bibliothèque électronique Taylor et Francis. page 13.

**L**

- **Lebres E. and Mouffouk F. (2008) .** Les cours Nationals d'hygiènes et de microbiologie des eaux de boisson. Manuel des travaux pratiques des eaux. Institut Pasteur d'Algérie. Algérie. 53-60p.
- **LiPuma and Currie, (2010) .**Trends Microbiol 18(2):64-66
- **Logan, (2012).** Mol Cell Probes 26(1):51-59
- **Loussert R et Brousse G. (1978).** L'olivier G.P. Maisonneuve et Larousse. France.PP. 1-127- 164- 283.

**M**

- **Madigan,(2018)**."Brock Biology of Microorganisms"15e édition, Pearson, 2018
- **Martín-Peláez, S., Martínez-González, M.A., Bes-Rastrollo, M., Fernández-Crehuet, M., Sánchez, V.M., Gea, A.(2021).** Olive oil consumption and risk of medication for hypertension in a Mediterranean cohort: The SUN study. Nutrients, 13(7), 2411
- **Maude,(2019).** Lignes directrices et normes pour l'interprétation des résultats analytiques en microbiologie alimentaire
- **Medina, E., Romero, C., Brenes, M., and de Castro,A.(2006).** Antimicrobial activity of olive oil, vinegar, and foods from their combination against plant pathogenic bacteria. Journal of Food Protection, 69(5), 1074-1078**.**
- **Metlef S. (2021).** Caractérisation et étude des activités antioxydantes et antibactériennes de l'huile d'olive algérienne. Thèse de doctorat en sciences alimentaires. Université de sidi bel abbès.Page 9-11; Page 28-31; Page 80; Page 85.
- **Moore, (2006)** .BMC Microbiol 6:104.
- **Mossel, D.A.A. (2003).** Essentials of the Microbiology of Foods.

**N**

- **N.Guezlane_tebibel ., B.kahlouche., S.Athmani Guemouri.(2008).**_microbiologie travaux pratique 2 éme année TCB et LMD.ed1.04.4973.
- **Niall A, Logan N, (2000**). Turnbull PCB. Bactéries aérobies sporulées. In: Freney J, Renaud F, Willy H, Bollet C, editors. « Précis de bactériologie clinique» ; éd ESKA. 2000. p. 965–81.
- **Nicoli, M. C. (2012).** Shelf life assessment of food. CRC Press..
- **Njoku, P.C. (2014).** Food Control, 46, 401-406
- **NRCP (2010).** Microbiological criteria for foods. National Research Council Plant.
- **Normes NF V76-122, 1994.**

**O**

- **Olivier, (2011).** LA PASTEURISATION, in: Opérations Unitaires En Génie Biologique.
- **Oyaizu et Komagata, (1983).** J Gen Appl Microbiol 29(1):17-24.

**P**

- **Pal, M., Gebara, A., and Ganaie, F. (2017).** Microbial contamination of common spices and spice mixtures in India. Nutrafoods, 16(4), 185-190.
- **Pechèr J.C., Acar J., Grenier B. and Nihoul E. (1982) .** Reconnaitre, comprendre et traiter les infections.4ème édition. Edisem ST-Hyacinthe. Québec.Canada.509 p
- **Philippe Dudez**, conserve traditionnelle et fermière, Edition Educagri.1998
- **Pioch, (1988).** Coagulase-negative Staphylococcus species in mixed fodder and on grain.
- **Pitt, J.I. and Hocking, A.D. (2009).** Fungi and Food Spoilage. Springer, New York.
- **Principles of Food Chemistry"** par John M. deMan, J.W. Finley, W.J. Hurst et C.R. Lee (4ème édition, Springer).
- **PUGLIESE A., LOIZZO M. R., TUNDIS R., O'CALLAGHAN Y., GALVIN K., - RUSSO V.** M.Production botanique et utilisations 2012.

**R**

- **Rejsek, (2002).** Analyse Des Eaux ; Aspects Réglementaires Et Techniques. Sceren.Paris. 360p.
- **Rodier J. (2005) .**L'analyse de l'eau. Eaux naturelles. Résiduaires. Eau de mer.8ème édition. Dunod. P :138-145.
- **Rodier J. (2009).** L'analyse de l'eau. 9ème édition. Dunod. Paris. 1579 p.
- **Romain A, Chloé C, Lucie C, Chloé D, Serge G, Joseph G, D SEDE, Juliette T, (2015).** Approche pédagogique du rôle des micro-organismes dans l'alimentation, Université de Lorraine, p8
- **Rowas S., Rahmani M., Elantari A., Idrissi D. J., Souizi A., Maata N. (2016).** Effect of geographical conditions (altitude and pedology) and age of olive plantations on the typicality of olive oil in Moulay Driss Zahroun. Mediterranean Journal of Biosciences. 1(3): 128-137.

**S**

- **Snyder HW, Sugie P (1986).** Pratiques d'analyse Microbiological-Sugie 2842
- **Soler C, Koeck JK (2004).** Sinusite maxillaire due à Bacillus licheniformis : à propos d'un cas survenu à Djibouti. Article accepté pour publication dans « médecine tropicale ».
- **Squillari, G., Mastrilli, V., Di Luca, A., Apone, F., Botta, A., Testa, G. et Sansone, L. (2023).** Propriétés antioxydantes de l'huile d'olive extra vierge : un examen complet de sa composition, de ses bienfaits pour la santé et de ses applications alimentaires. Antioxydants, 12(2), 290
- **S. Suzanne Nielsen, 5th edition, Springer, 2010). "**Food Analysis
- **Stanier . (1966) .**J Gen Microbiol 43(1):159-271

**T**

- **Tafakori, V. (2015).** European Journal of Lipid Science and Technology, 117(5), 679-688.

- **Techniques d'analyse et de contrôle dans les industries agroalimentaires"** par G. Linden et D. Lorient (Tec et Doc Lavoisier).
- **Tellez- pérez (2013),** Effect of İnstant Effect of Instant Controlled Pressure Drop Process Coupled to Drying and Freezing on Antioxidant Activity of Green “Poblano” Pepper (Capsicum annuum L.)

**V**

- **Veillet S. (2010).** Enrichissement nutritionnel de l’huile d’olive : Entre Tradition et Innovation. these de doctorat en Sciences de l’Université d’Avignon et des Pays de Vaucluse Academie d’aix-marseille. P151. P153.
- **Vij, V., Govender, P., Naicker, B., and Bignell, L. (2006**). Enumeration of Coliforms from Ready-to-Eat Foods. International Journal of Food Microbiology, 108(1), 145-151.

**Z**

- **Zribi, A. (2015).** Nutrient/chemical scoring and CATA-analysis of traditional and industrial tunisian harissa. Journal of Ethnic Foods, 2(2), 81-88.

## Websites


**[1]** https://www.lesfruitsetlegumesfrais.com/fruits-legumes/legumes-fruits/tomate (consulted on 11.04.2024 at. 09 :10).

**[2]** http://www.djamiatic.net/tomate/cours/cours6.htm (consulted on 11.04.2024 at 11:15 am).

**[3]** https://www.piccantino.com/info/magazine/harissa-paste-recipe( consulted on 18.04.2024 at 9 :30).

**[4]** https://www.babeloued.fr/blogs/conseils/harissa-origine (CONSULTED ON 19.04.2024 at 13 :05).

**[5]** https://www.sofrabrick.com/2022/03/22/harissa-et-son-histoire/)22 mars 2022 - 14 h 40 min consultez le 19.04.2024 à 13 :30

**[6]** https://www.cuisineaz.com/articles/harissa-2440.aspx) by Stéphanie Bartczak Published on 01/12/2018, updated on 19/09/2019 at 11:54 ( consulted on 19.04.2024 at 13 :30).

**[7]** https://minimalistbaker.com/easy-diy-harissa-paste/ (consulted on 19.04.2024 at 14 :00).

**[8]** (https://tpe-el-piment.webnode.page/le-piment-kesako/un-peu-de-chimie-les-composants-du-piment/)TPE 2009/2010( Consulted on 19.04.2024 at 14 :15).

**[9]** https://tpe-el-piment.webnode.page/le-piment-kesako/un-peu-de-chimie-les-composants-du-piment/( consulted on 19/04/2024 at 14:17).

**[10]** https://www.goodepices.com/fichier-produit/5371-102126-harissa-la-flamme-du-ca-t0000088404.pdf.html (consulted on11/04/2024 at 15:08)**.**

**[11]** https://cuisine.journaldesfemmes.fr/encyclopedie-produits/2725075-harissa/#:~:text=Ce%20piment%20poss%C3%A8de%20beaucoup%20de,pas%20avoir%20l'estomac%20fragile (consulted on 11/04/2024 at 15:10).

**[12]** https://www.cuisineaz.com/articles/harissa-2440.aspx consulted on 11/04/2024 at 15:15

**[13]** https://alimentation.pagesjaunes.fr/astuce/voir/620927/harissa (consulted on 20/04/2024 at 17:00).

**[14]** https://santeenafrique.com/nutrition-sauce-harissa-un-savoir-faire-tunisien/ (consulted on 20/04/2024 at 17:10).

**[15]** https://www.cuisineaz.com/articles/harissa-2440.aspx By Stephanie Bartczak Published on 01/12/2018, updated on 19/09/2019 at 11:54

**[16]** https://www.unlockfood.ca/fr/Articles/Technologie-culinaire/Biotechnologie/Nouveaux-aliments/Agents-de-conservation.aspx (consulted on 19/04/2024 at 18 :00).

**[17]** https://www.avenirsantemutuelle.fr/ma-sante-et-moi/thema-sante/conservation-des-aliments-un-enjeu-de-sante-publique/ last update 24/05/2022 consulted on 21/04/2024 at 14:00

**[18]** https://www.avenirsantemutuelle.fr/ma-sante-et-moi/thema-sante/conservation-des-aliments-un-enjeu-de-sante-publique( consulted on 21/04/2024 at15:00).

**[19]** https://www.cuisineaz.com/articles/harissa-2440.aspx) by Stéphanie Bartczak Published on 01/12/2018, updated on 19/09/2019 at 11:54( consulted on 21/04/2024 at 15 :10).

**[20]** https://www.babeloued.fr/blogs/conseils/harissa-tunisie 14 novembre 2022 (consulted on 21/04/2024 at 23:00).

**[21]** Conseil Oléicole International (2001) Olivae 87 : 22-24

**[22]** N. Benyahia; K. Zein. Analyse des problèmes de l'industrie de l'huile d'olive et solutions récemment développées. Contribution spéciale de Sustainable Business Associates (Suisse) à l'atelier «Pollution and Development issues in the Mediterranean Basin» du 28 janvier 2003 dans le cadre de la 2ème Conférence Internationale Swiss Environmental Solutions for Emerging Countries (SESEC II) du 28-29 janvier 2003 à Lausanne, Suisse

**[23]** https://www.quechoisir.org/guide-d-achat-huiles-d-olive-n3751/#l-origine (consulted on17/05/2024 at 17:11).

**[24]** https://www.passeportsante.net/huiles-vegetales-g152/Fiche.aspx?doc=huile-olive (consulted on 17/05/2024 at 17 :14).

**[25]** https://www.lanutrition.fr/conserver-les-aliments-dans-lhuile-mode-demploi#:~:text=Il%20est%20recommand%C3%A9%20d'utiliser,huile%20de%20colza%2C%20meilleur%20march%C3%A9( Consulted 17/05/2024 at 22 :00).

**[26]** https://www.healthline.com/nutrition/11-proven-benefits-of-olive-oil#TOC_TITLE_HDR_12 (consulted on 17/05/2024 at 15:33).

**[27]** https://www.huiledoliveitalienne.com/epicerie-fine/specialites-a-l-huile/( consulted on 17/05/2024 at 21:30).

**[28]** https://fr.openfoodfacts.org/produit/6133991001542/zimba (cosulted on 21/05/2024 at 23:59).

**[29]** http://www.made-in algeria.com/vitrine/art_detail.php?pc1=l6mRqaY=&div=1&tc1=lKySnKeY# (consulted on 2105.2024 à 12 :03

**[30]** Haddad A, Gourid R. Procédé de fabrication de confiture d'orange et contrôle de qualité. (Conserverie ABIDI), Institut de Formation Professionnelle Et De l'éducation Amri BoudjamaaSkikda, 2018-2019.

**[31]** www.theirrysouccar.com (consulted on 01/05/2024 at 15 :00).

**[32]** Balance de précision : choix et méthode de pesée (lachimie.fr)

**[33]** https://www.jumo.net/attachments/JUMO/attachmentdownload?id=3633( consulted on 01/05/2024 at 16 :00).

**[34]** Http://www.fao.org/3/y1579f/y1579f02.htm(consulted on 01/05/2024 at 17:00).

**[35]** http://www.kruess.com/ (consulted on 01/05/2024 at 17 :15).

**[36]** https://inrs.fr MetroPol-Methode-Analyse-METHANALYSE_91.pdf

**[37]** inrs.fr (consulted on 02/05/2024 at 15 :08).

**[38]** https://microbiologie-clinique.com/api-for-microorganism-identification.html (consulted on 10/05/2024at 4:27).

**[39]** https://www.ceaeq.gouv.qc.ca/methodes/pdf/ma115cond11.pdf (consulted on 16/05/2024 at 10:15 minutes).

**[40]** Board B.W. Le contrôle de la qualité dans l'industrie du traitement des fruits et légumes. Etude F.A.O., alimentation et nutrition n°39. p 75, 1987.

**[41]** FAO/OMS. (2009). Code d'usages pour l'hygiène des épices et des plantes aromatiques déshydratées. http://www.fao.org/input/download/standards/27/CXP_042f.pdf (consulted on 18/05/2024 at 16:00).

**[42]** Santé Canada. (2012). Ligne directrice et norme de rendement sur les coliformes : définition et mode d'échantillonnage. https://www.canada.ca/fr/sante-canada/services/salubrite-aliments-milieu-travail-residences/rapports-publications/salubrite-aliments/ligne-directrice-norme-rendement-coliformes-definition-echantillonnage.html http://www.fao.org/input/download/standards/27/CXP_042f.pdf (consulted on 13/05/2024 at 21:00).

**[43]** Jonathan, 2021. Intoxication alimentaire à staphylocoque - Troubles digestifs - Manuels MSD pour le grand public [WWW Document]. URL https://www.msdmanuals.com/fr/accueil/troubles(consulted on 18/05/2024 at 15 :00).

**[44]** digestifs/gastroent%C3%A9rite/intoxication-alimentaire-%C3%A0-staphylocoque (accessed 5.21.23).

**[45]** FAO. (2020). Les régimes alimentaires sains et la nutrition: Des politiques aux actions suivies de leur évaluation. Organisation des Nations Unies pour l'alimentation et l'agriculture. https://www.fao.org/3/ca9508fr/CA9508FR.pdf (consulted on 17/04/2024/ at 8:00).

**[46]** Organisation mondiale de la Santé (2021). Principes et directives pour la gestion des risques microbiologiques (No. OMS/PBD/Risques microbiologiques/21.07). Organisation mondiale de la Santé. https://apps.who.int/iris/handle/10665/342365 (consulted on 15/04/2024 at 21:00).

**Abstract**

In this study, we evaluated the evolution of the quality of a food product (harissa) preserved in olive oil for a period of one month. The objective was to analyze the impact of olive oil on the maintenance of desirable microbiological and physicochemical characteristics during conservation.

For the physicochemical quality, various key parameters were monitored: (pH, conductivity, degree of Brix, titratable acidity, Ash content) the results showed that the values of certain parameters are within the standards according to the codex alimentarius.

On the microbiological level, the results showed a low microbial growth in the samples preserved with olive oil compared to the controls without oil, due to the antimicrobial effect of the phenolic compounds. The total absence of pathogenic germs in the four samples with or without olive oil.

After 1 month, the samples preserved in olive oil therefore had a better overall quality profile with a microbial load and a level of physicochemical alteration significantly lower than the controls. Olive oil has therefore proven to be an excellent natural preservative for this food product.

**Key words**: physico-chemical analysis, microbiological analysis, harissa, conservation, olive oil.

**Résumé**

Dans cette étude, nous avons évalué l'évolution de la qualité d'un produit alimentaire (harissa) conservé dans de l'huile d'olive pendant une période d'un mois. L'objectif était d'analyser l'impact de l'huile d'olive sur le maintien des caractéristiques microbiologiques et physico-chimiques souhaitables pendant la conservation.

Pour la qualité physico-chimique, différents paramètres clés ont été suivis: (pH, conductivité, degré de Brix, acidité titrable, Teneur en cendres) les résultats ont montré que les valeurs de certains paramètres sont dans les normes selon le codex alimentarius.

Sur le plan microbiologique, les résultats ont montré une faible croissance microbienne dans les échantillons conservés avec de l'huile d'olive par rapport aux témoins sans huile, en raison de l'effet antimicrobien des composés phénoliques. L'absence totale de germes pathogènes dans les quatre échantillons avec au sens l'huile d'olive.

Après 1 mois, les échantillons conservés dans l'huile d'olive présentaient donc un meilleur profil de qualité global avec une charge microbienne et un niveau d'altération physico-chimique significativement inférieurs aux témoins. L'huile d'olive s'est donc avérée être un excellent conservateur naturel pour ce produit alimentaire.

**Mots clés** : analyse physico-chimique, analyse microbiologique, harissa, conservation, huile d'olive.

**ملخص**

في هذه الدراسة، قمنا بتقييم تطور جودة المنتج الغذائي (الهريسة) المحفوظ في زيت الزيتون لمدة شهر واحد. كان الهدف هو تحليل تأثير زيت الزيتون على الحفاظ على الخصائص الميكروبيولوجية والفيزيائية الكيميائية المرغوبة أثناء الحفظ.

بالنسبة للجودة الفيزيائية والكيميائية، تم رصد العديد من المعلمات الرئيسية: (الرقم الهيدروجيني، الموصلية، درجة البركس، الحموضة القابلة للمعايرة، محتوى الرماد) أظهرت النتائج أن قيم بعض المعلمات تقع ضمن المعايير وفقا للدستور الغذائي.

على المستوى الميكروبيولوجي، أظهرت النتائج نموا ميكروبيا منخفضا في العينات المحفوظة بزيت الزيتون مقارنة بالضوابط بدون زيت ، بسبب التأثير المضاد للميكروبات للمركبات الفينولية. الغياب التام للجراثيم المسببة للأمراض في العينات الأربع مع أو بدون إضافة زيت الزيتون.

بعد شهر1، كانت العينات المحفوظة في زيت الزيتون ذات جودة شاملة أفضل مع حمل ميكروبي ومستوى من التغيير الفيزيائي الكيميائي أقل بكثير من عناصر التحكم. لذلك أثبت زيت الزيتون أنه مادة حافظة طبيعية ممتازة لهذا المنتج الغذائي.

**الكلمات المفتاحية**: التحليل الفيزيائي الكيميائي، التحليل الميكروبيولوجي، الهريسة ، الحفظ ، زيت الزيتون.

# Annex

## Annex 01: Media used

**Chapman medium: pH = 7.4**

| | |
|---|---|
| Bacteriological peptone | 10g |
| Beef meat extract | 1g |
| Sodium chloride | 75g |
| Mannitol | 10g |
| Phenol red | 0.025g |
| Agar | 15g |
| Distilled water | 1000ml |

**Cetrimide Agar: pH = 7.1**

| | |
|---|---|
| Gelatin peptone | 16 g/l |
| Casein peptone | 10 g/l |
| Tetradonium bromide (cetrimide) | 0.2 g/l |
| Nalidixic acid | 15 mg/l |
| Potassium sulfate | 10 g/l |
| Magnesium chloride | 1.4 g/l |
| Agar | 10 g/l |
| Distilled water | 1000 m |

**Preparation of Meat liver Agar (VF):pH = 7.2**

*Basic agar :

| | |
|---|---|
| Base meat liver | 30g |
| Glucose | 2g |
| Starch | 2g |
| Agar | 11g |
| Distilled water | 1000ml |

*Complete agar :

| | |
|---|---|
| Same formula as the base medium to which are added : | |
| 5% Sodium sulfite | 50ml |
| Ammoniacal iron alum at | 5%10ml |

**Hektoen Agar: pH = 7.5**

| | |
|---|---|
| Proteose peptone | 12g |
| Yeast extract | 3g |
| Sodium chloride | 5g |
| Sodium thiosulfate | 5g |
| Bile salts | 9g |
| Ammoniacal iron citrate | 1.5g |
| Salicin | 2g |
| Lactose | 12g |
| Sucrose | 12g |
| Fuchsin acid | 0.1g |
| Bromothymol blue | 0.065g |
| Agar | 14g |
| **PCA Medium : pH = 7** | |
| Trypsic casein hydrolysate | 5g |
| Yeast extract | 2.5g |
| With Glucose | 1g |
| The Agar agar | 15g |
| Distilled water | 11ml |