People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
A. MIRA University, BEJAIA
Faculty of Natural and Life Sciences
Department of Food Sciences
Laboratory or research unit attached to it: Laboratory of Biomathematics, Biophysics, Biochemistry and Scientometry (L3BS)

THESIS

Presented by
HIMED-IDIR Hayat

In order to obtain the diploma of
DOCTORATE OF SCIENCES

Field: Natural and Life Sciences **Sector:** Biology
Speciality: Food Sciences

Theme

INCORPORATION OF ROSEMARY (*ROSMARINUS OFFICINALIS*) IN FERMENTED MILK AND FRESH CHEESE: IMPACT ON THE PHYSICOCHEMISTRY OF DAIRY PRODUCTS AND THE PHYTOCHEMISTRY OF THE PLANT

Supported on: …… **In front of the Jury composed by:**

Name and First Name	**Grade**		
Mr. MADANI Khodir	Professor	Univ. of Bejaia	President
Mrs. BOULEKBACHE Lila	Professor	Univ. of Bejaia	Reporter
Mrs. CARDOSO M. Susana	MCA	Univ. of Aveiro-Portugal	Co-Reporter
Mrs. OUCHEMOUKH Nadia	MCA	Univ. of Bejaia	Examiner
Mr. KADRI Nabil	MCA	Univ. of Bouira	Examiner
Mr. KECHBAR Mohamed Seif Allah	MRA	CRSTRA of Biskra	Examiner

University Year: 2020/2021

République Algérienne Démocratique et Populaire
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
Université A.MIRA-BEJAIA
Faculté des Sciences de la Nature et de la Vie
Département des Sciences Alimentaires
Laboratoire ou unité de recherche de rattachement : Laboratoire de Biomathématique, Biophysique, Biochimie et Scientométrie (L3BS)

THÈSE

Présentée par

HIMED-IDIR Hayat

Pour l'obtention du grade de
DOCTEUR EN SCIENCES

Domaine : Sciences de la Nature et de la Vie **Filière : Biologie**
Option : Sciences Alimentaires

Thème

INCORPORATION DU ROMARIN (*ROSMARINUS OFFICINALIS*) DANS LE LAIT FERMENTE ET LE FROMAGE FRAIS: IMPACT SUR LA PHYSICOCHIMIE DES PRODUITS LAITIERS ET LA PHYTOCHIMIE DE LA PLANTE

Soutenue le :…….. **Devant le Jury composé de :**

Nom et Prénom	Grade		
Mr. MADANI Khodir	Professeur	Univ. de Bejaia	Président
Mrs. BOULEKBACHE Lila	Professeurr	Univ. de Bejaia	Reporteur
Mrs. CARDOSO M. Susana	MCA	Univ. de Aveiro-Portugal	Co-Reporteur
Mrs. OUCHEMOUKH Nadia	MCA	Univ. de Bejaia	Examinatrice
Mr. KADRI Nabil	MCA	Univ. de Bouira	Examinateur
Mr. KECHBAR Mohamed Seif Allah	MRA	CRSTRA de Biskra	Examinateur

Année universitaire: 2020/2021

Acknowledgments

First, my most sincere gratitude to our Lord, Allah, for giving me the opportunity to undertake this study and for giving me the strength to complete it.

The accomplishment of this work could not have been achieved without the support of many people to whom I would like to express my gratitude and sincere thanks.

This work carried out in the Research Laboratory of Biomathematics, Biophysics, Biochemistry and Scientometry (L3BS), Faculty of Natural and Life Sciences, University of Bejaia-Algeria-under the direction of ***Prof. Lila BOULEKBACHE-MAKHLOUF****. I would like to express my gratitude to her for the efforts and support she has shown me throughout this thesis, her scientific skills, her encouragement and the trust she has given me, which have enabled me to carry out this study.*

I would like sincerely thank my co-director ***Dr. Susana M. Cardoso****, of the Organic Chemistry, Natural Products and Food Stuffs (QOPNA) Research Unit for welcoming me and for doing me the honor of leading part of this work. I am grateful to her for the availability, generosity, trust and permanent support she showed me during my stay in Aveiro-Portugal.*

To my president of the jury, ***Prof. Khoddir Madani.****, I am filled with the immense honor that you do me by agreeing to chair the jury of my thesis and examining this work, please find here the expression of my sincere gratitude.*

Dr. OUCHEMOUKH Nadia*, thansks for agreeing to be part of the jury for my thesis and for the remarks and corrections, you will make. Please find here the expression of my sincere gratitude.*

My most sincere thanks also go to ***Dr. Nabil Kadri*** *for agreeing to be part of the jury for this thesis and for the remarks and corrections, you will make. Please accept Sir, my deepest respect.*

I would like to express my deepest consideration and respect to ***Dr. Mohamed Seif Allah Kechbar****, director of the CRSTRA-Biskra Research Center, for having accepted to judge this work despite all his responsibilities.*

I am also deeply grateful to my parents and al my family for their appreciable help, their support, encouragement and guidance during all my years of study.

The word "Thank you" is very short to express my deep gratitude to my dear husband, first as a friend and husband, for his moral support, his love, his listening, and his precious advice. He knew how to push me in my choices and always encouraged me to go further, no matter how much it costs us. Thank you, also, for your good picking of the plant "Rosemary" which served as the plant matrix of this thesis.

My warmest thanks also go to my family in-law for their encouragement and help especially my mother-in-law.

I thank my angel boy "Dany" for his perception, his love and his courage during all my 6 years of doctoral study. Dear son, you are my reason for being.

I am very grateful to all the members of Biomathematics, Biophysics, Biochemistry and Scientometry (L3BS) laboratory: to teachers and doctoral colleagues for the good times, the atmosphere and the joys spent together and to engineers and technicians for their help, their kindness on a daily basis, their support and thanks for their friendship.

Thanks also for technicians from Food Technology and Sensory Analysis Laboratories, for their help and service.

I would like to express my gratitude to all those whom helped me at the Department of Chemistry from the University of Aveiro, Portugal (Marcelo Catarino, Lydia Saidi et Monica Valega, Joana Coelho, Catarina Almeida, Ricardo Ferreira and Andria Selva).

I express my appreciations to the managers of the "QualiLab Laboratory" and "DANONE dairy" for accepting me to carry out part of my thesis.

My feelings of gratitude also go to all my friends and colleagues for their nice moments shared together (Zakia, Khokha, Wassila, Awetef, Keltoum, Fayza, Fatima, Najet, Ibtissam, Hocine, Nour El-Islam, Chawki ...)

My thoughts for the whole Division/Team: Phœniciculture, biotechnology and valuation of date palm products and by-products

Finally, I would like to express my gratitude to all those whom I was unable to mention and who contributed directly or indirectly to the success of this work.

PUBLICATIONS AND PRESENTATIONS RELATED TO THE THESIS

• Published papers :

1. Original article: Effect of rosemary (*Rosmarinus officinalis* L.) supplementation on fresh cheese: Physicochemical properties, antioxidant potential and sensory attributes. **Himed-Idir Hayat**, Mouhoubi Khokha, Siar El-hocine, Boudries Halim, Hafid Mansouri, Adjeroud, Nawel, MadaniKhodir, Boulekbache-Makhlouf Lila*. Journal of Food Processing and Preservation (2020). *https://doi.org/10.1111/jfpp.15057*

2. Article to submit: *Rosmarinus Officinalis L.* Leaves as a Bioactive Cheese Ingredient. **Himed-Idir Hayat**, Boulekbache-Makhlouf Lila, Madani Khodir, Artur M. S. Silva, Susana M. Cardoso*.

• Communications :

1. Himed-Idir Hayat, Bouderies Halim, Madani Khodir et Boulekbache Lila. Incorporation des feuilles de *Rosmarinus officinalis* dans un fromage frais ainsi que l'étude de leur effet sur la flore lactique et quelques paramètres physicochimiques et sensoriels. International seminar on local products: a tool for the development of Mountain Agriculture, 15 and 16 December 2018. Chemini, Bejaia, Algeria.

2. HIMED-IDIR Hayat, MOUHOUBI Khokha, DJAOUD Kahina, BOULEKBACHE-MAKHLOUF Lila, CARDOSO Susana et MADANI Khodir. Activités Biologiques De L'extrait Phénolique Des Feuilles De *Rosmarinus officinalis* L. National Exchange Seminar: At the Interfaces of Sustainable Development. 20 and 21 December 2017, Bejaïa, Algeria.

Abstract

Rosmarinus officinalis L. leaves can be valorized by their uses in the production of healthy foods. Dry rosemary leaves powder and its lyophilized ethanolic extract at different concentrations (g/100 g) were used for the formulation of fresh cheeses. This study evaluated whether or not this fortification could affect some physicochemical characteristics, phenolic composition and antioxidant capacity of fresh cheeses along storage period at 5°C. The ethanolic extract was obtained under the optimum conditions of microwave-assisted extraction (MAE) using response surface methodology (RSM) coupled with a Box–Behnken design (BBD). The optimum conditions were as follows: ethanol concentration 78.162%, microwave power 351.825W, ratio of solvent to raw material 101.623:1, and extraction time 122.648 s. Results showed that this enrichment did not affect physicochemical parameters (pH, aciity, proteins, fat) of the formulated cheeses while its effect on cheese color parameters was more marked. Moreover, this incorporation has increased substantially values of total phenolic content (TPC) and ABTS scavenging activity during storage period compared to the control cheese. UHPLC-DAD-ESI-MS^n analysis revealed the presence of rosmarinic acid (RA), rosmanol (R), carnosic acid (CA), carnosol (C) and methoxy-carnosic acid (MCA) in rosemary extract and enriched cheeses. Principal components analysis (PCA) was performed and results confirmed a strong relationship between antioxidant activity and these phenolic compounds. Additionally, a correlation between physicochemical parameters, antioxidant activity as well as the sensory characteristics was carried out. Cheeses fortified with 0.5%, 0.75% of powder and 0.5% of extract, were most appreciated (60%–80%) by the panelists. In conclusion, the consumers can successfully employ rosemary as dairy supplements as it improves markedly the TPC and the antioxidant activity of cheese as well as a distinctive taste, aroma and acts as flavor enhancer for better acceptability. In this study rosemary extract and whey protein complexation was highlighted by the turbidity test. It was concluded that rosemary phenolic acids were responsible of this complexation as complexes were detectable at 400nm.

Key words: *Rosmarinus officinalis L.*, microwave-assisted extraction, phenolic compounds, sensory evaluation, supplemented cheese, functional food.

Résumé

Les feuilles de *Rosmarinus officinalis* L. peuvent être valorisées par leurs utilisations dans la production d'aliments bénéfiques pour la santé. La poudre de feuilles de romarin séchées et son extrait éthanolique lyophilisé à différentes concentrations (g / 100 g) ont été utilisées pour la formulation de fromages frais. Cette étude a évalué si cette fortification pouvait ou non affecter certaines caractéristiques physico-chimiques, la composition phénolique et la capacité antioxydante des fromages frais pendant la période de stockage à 5 ° C. L'extrait éthanolique a été obtenu dans les conditions optimales d'extraction assistée par micro-ondes (MAE) en utilisant la méthodologie de surface de réponse (RSM) couplée à un design Box-Behnken (BBD). Les conditions optimales étaient les suivantes: concentration d'éthanol 78,162%, puissance micro-onde 351,825W, rapport solvant / matière première 101,623: 1 et temps d'extraction 122,648 s. Les résultats ont montré que cet enrichissement n'affectait pas les paramètres physico-chimiques (pH, acidité, protéines, lipides) des fromages formulés alors que son effet sur les paramètres de couleur du fromage était plus marqué. De plus, cette incorporation a augmenté sensiblement les valeurs de la teneur phénolique totale (TPC) et de l'activité de piégeage ABTS pendant la période de stockage par rapport au fromage témoin. L'analyse UHPLC-DAD-ESI-MSn a révélé la présence d'acide rosmarinique (RA), de rosmanol (R), d'acide carnosique (CA), de carnosol (C) et d'acide méthoxy-carnosique (MCA) dans l'extrait de romarin et les fromages enrichis. L'analyse en composantes principales (ACP) a été réalisée et les résultats ont confirmé une forte relation entre l'activité antioxydante et ces composés phénoliques. De plus, une corrélation entre les paramètres physico-chimiques, l'activité antioxydante ainsi que les caractéristiques sensorielles a été réalisée. Les fromages enrichis de 0,5%, 0,75% de poudre et 0,5% d'extrait ont été les plus appréciés (60% -80%) par les panélistes. En conclusion, les consommateurs peuvent utiliser avec succès le romarin comme suppléments laitiers car il améliore nettement le TPC et l'activité antioxydante du fromage ainsi qu'un goût et un arôme distinctifs et agit comme exhausteur de goût pour une meilleure acceptabilité. Parmi les méthodes de complexations des polyphénols du romarin et les protéines sériques du lait, le test de turbidité a été mis en évidence. Il a été conclu que les acides phénols du romarin sont responsables de cette complexation détectée à 400nm.

Mots clés: *Rosmarinus officinalis* L., extraction assistée par micro-ondes, composés phénoliques, évaluation sensorielle, fromage supplémenté, aliment fonctionnel.

ملخص

يمكن تقييم أوراق إكليل الجبل من خلال استخدامها في إنتاج الأطعمة الصحية. تم استخدام مسحوق أوراق إكليل الجبل المجففة وخلاصة الإيثانول المجففة بتركيزات مختلفة لتكوين الأجبان الطازجة. قيمت هذه الدراسة ما إذا كان هذا التحصين يمكن أن يؤثر على بعض الخصائص الفيزيائية والكيميائية والتركيب الفينولي والقدرة تم الحصول على المستخلص الإيثانولي في ظل الظروف المثلى للاستخراج بمساعدة المضادة للأكسدة للأجبان الطازجة خلال فترة التخزين في 5 درجات مئوية كانت الظروف المثلى كما يلي: تركيز الإيثانول 78.162٪ ، طاقة الميكروويف 351.825 واط ، نسبة باستخدام منهجية سطح الاستجابة (MAE) الميكروويف أظهرت النتائج أن هذا التخصيب لم يؤثر على المتغيرات الفيزيائية والكيميائية (الأس المذيبات / المواد الخام 101.623: 1 ووقت الاستخراج 122.648 ثانية الهيدروجيني والحموضة والبروتينات والدهون) للأجبان المصنعة بينما كان تأثيره على معاملات لون الجبن أكثر وضوحًا. بالإضافة إلى ذلك ، أدى هذا الدمج إلى UHPLC-DAD-ESI-MSn كشف زيادة كبيرة في قيم المحتوى الفينولي الكلي ونشاط الكسح خلال فترة التخزين مقارنة بجبن التحكم
ن وجود حمض الروزمارينيك ، روزمانول ، حمض الكرنوسيك ، كارنوزول وحمض ميثوكسي كارنوسيك في خلاصة إكليل الجبل والجبن المدعم
تم إجراء تحليل المكون الرئيسي وأكدت النتائج وجود علاقة قوية بين نشاط مضادات الأكسدة وهذه المركبات الفينولية. بالإضافة إلى ذلك ، تم إجراء علاقة ارتباط بين المعلمات الفيزيائية والكيميائية والنشاط المضاد للأكسدة وكذلك الخصائص الحسية. كانت الجبن المخصب بنسبة 0.5٪ ، مسحوق 0.75٪ وخلاصة 0.5٪ هي في الختام ، يمكن للمستهلكين استخدام إكليل الجبل بنجاح كمكمل لمنتجات الألبان لأنه يحسن بشكل ملحوظ من الأكثر شيوعًا (60٪ -80٪) من قبل أعضاء اللجنة .نشاط الإنعاش القلبي الرئوي ومضادات الأكسدة للجبن بالإضافة إلى المذاق والرائحة المميزة ويعمل كمحسن للنكهة للحصول على قبول أفضل

الكلمات الأساسية: إكليل الجبل، الاستخلاص بمساعدة الميكروويف، المركبات الفينولية، التقييم الحسي، الجبن المكمل، الغذاء الوظيفي

TABLE OF CONTENTS

List of tables

List of figures

General introduction……..……..**1**

Literature review

CHAPTER I – General informations on rosemary

I.1. The Labiatae family (Lamiaceae)…….….**4**

I.2. Brief history of rosemary…….....….**4**

I.3. Botanical description, distribution and cultivation…….....**5**

I.4. Nutritional composition (macro and micronutrients)…….…...**7**

I.5. Rosemary secondary metabolites…….....**9**

I.5.1 Phenolic compounds……...…..**10**

I.5.2. Essential oils……...….**12**

I.5.3 Alkaloids……..…….**13**

I.6. Rosemary biological activities……..……..**13**

I.6.1. Antimicrobial activity…….……**13**

I.6.2. Antioxidant activity…….**14**

I.6.3. Anti-inflammatory activity……..……**14**

I.6.4. Antiseptic activity……...…….**15**

I.6.5. Rosemary medicinal uses…….....**15**

I.7. Uses of rosemary extracts in foods…….**17**

I.7.1. Function in foods…….**17**

I.7.2. Dietary Exposure…….……**18**

CHAPTER II - General information on fresh cheese and polyphenols-proteins interactions

II.1. Cheese production and consumption…….**20**

II.2. Description of cheese…….….**21**

II.2.1. Coagulation of milk…….**23**

II.2.2. Starter-free fresh cheese…….….**24**

II.2.3. Fresh cheese manufacturing……...……**25**

II.2.4. Fresh cheese types……..…**26**

II.3. Polyphenol-Protein Interactions……..……..**26**

II.3.1. Factors affecting the interactions between proteins and phenolic compounds……..**28**

Experimental part

FIRST PART: Phytochemical analysis of Rosmary

CHAPTER I: MATERIALS AND METHODS

I. Plant material……32
II. Optimization of microwave-assisted extraction (MAE)……32
II.1. Spectrophotometric determination of phenolic compounds of rosemary extract.....35
III. UHPLC-DAD-ESI-MS^n analysis……35
IV. Biological analyses of rosemary leaves extract……36
IV.1. Antioxidant capacity……36
IV.1.1. DPPH radical scavenging capacity……36
IV.1.2. Ferric reducing antioxidant power (FRAP) assay……37
IV.1.3. Nitric oxide (NO) Scavenging assay……38
IV.1.4. Scavenging effect on superoxide radical (nonenzimatic assay)……38
IV.2. Inhibition of enzymes……39
IV.2.1. Inhibition of alpha glucosidase enzyme……39
IV.2.2. Assay of Lipase inhibitory activity……40
V. Statistical analysis……40

CHAPTER II: RESULTS AND DISCUSSION

I. Moisture and ash content of rosary leaves……41
II. Optimization of microwave-assisted extraction……41
III. Rosemary phenolics and characterization of phenolic compounds……45
IV. Biological activities of rosemary extract……47
IV.1. Antioxidant activity……47
IV.2. Antidiabetic activity……50

SECOND PART: Enrichment of cheese by rosmary

CHAPTER I: MATERIALS AND METHODS

I. Impact of rosemary addition on physicochemical parameters, sensory attributes and microbiological quality of rosemary-supplemented cheese……52

I.1. Preparation of rosemary-supplemented cheeses and sampling……52
I.2. Physicochemical and compositional analyses……52
I.3. Microbiological Analysis……53
I.4. Sensory analyses……54

II. Effect on color, phenolic compounds and antioxidant activity of rosemary fortified fresh cheese ……..…..…**55**

II.1. Preparation of rosemary-supplemented cheeses and obtaining of the respective extracts……..**55**

II.2. Surface color……..……..……**55**

II.3. Quantitative and qualitative analysis of phenolic compounds ……..…….**56**

II.4. Antioxidant capacity of cheese samples…….……**56**

III. Statistical analysis……..…..…**56**

CHAPTER II: RESULTS AND DISCUSSION

I. Physicochemical parameters of cheese extracts……**58**

I.1. Rosemary-cheese supplementation effect on pH, acidity, proteins and fat contents…….……**58**

I.2. Surface color evolution……..…**60**

I.3. Total phenolic content as affected by rosemary enrichment……...…**62**

I.4. Antioxidant capacity……..….**63**

II. Microbiological Analysis……..…...…**65**

III. Sensory analysis (hedonic test)……**67**

IV. Relationship between physicochemical parameters, sensory attributes, phenolics and antioxidant capacity of cheese samples…….……**69**

***THIRD PART: Enrichment of w*hey milk by rosmary**

CHAPTER I: MATERIALS AND METHODS

I. Whey milk-rosemary and whey milk proteins content……..…**73**

I.1. Samples preparation……..……..**73**

I.2. Protein content…….....……**73**

II. Turbidity test……...…..**73**

III. Determination of phenolic compounds in milk – rosemary samples……**74**

CHAPTER II: RESULTS AND DISCUSSION

I. Determination of protein in milk whey……....……**75**

II. Turbidity test……..…..**75**

II.1. Turbidity test of the rosemary extract-whey proteins mixture……..……**75**

II.2. Turbidity test of the caffeic acid-whey protein mixture…….……**77**

III. Determination of total poyphenos in the whey filtrate of mik…….…....**78**

GENERAL CONCLUSIONS AND PERSPECTIVES……...…**80**

References ……**82**

List of abeviations

BBD: Box-Behnken Design

BDA : Banca Dati di Composizione degli Alimenti per Studi Epidemiologici;

BEDCA : Base de Datos Espa~nola de Composici_on de Alimentos

BSA : Bovine serum albumin

C.V: Coefficient of variation

C: carnosol

CA: carnosic acid

DF: Degree of freedom

DII : Dairy Industries International

DPPH: 2,2-diphenyl 1-picrylhydrazyl reagent

GAE : Gallic Acid Equivalents

IC50 : Inhibitory concentration at 50%

MAE: Microwave assisted extraction

MCA: 1, 2, methoxy-carnosic acid

NADH: Nicotinamide adenine dinucleotide

NBT: Nitro blue tetrazolium

NO : Nitric oxid

PCA: Principal components analysis

PMS: Phenazonium methosulfate

PNPG: 4-nitrophenyl α-D-glucopyranoside

RA: rosmarinic acid

RSM: Response surface methodology

SO : Superoxyd

TF: total flavonoids

TPC: total phenolic content

UHPLC: Ultra-high performance liquid chromatography coupled to electrospray mass spectrometry

USDA: United States Department of Agriculture

List of tables

Table I: Nutritional value of rosemary plant……**8**

Table II: Minerals content (mg/100 g) of rosemary……..……..**9**

Table III : Nutritional value of fresh cheese……..……..**25**

Table IV: Experimental levels of the factors used in BBD for the MAE…….……..**33**

Table V: Box-Behnken design for total phenolic compounds of rosemary leaves obtained by microwave-assisted extraction ……..**34**

Table VI: Moisture and ash content of rosemary leaves……**41**

Table VII: Analysis of variance (ANOVA) for the experimental results obtained by microwave-assisted extraction…….…...**42**

Table VIII: Compounds characterized in rosemary-leaf extract……..**...45**

Table VIIII9: Antioxidant activities of rosemary extract and standards expressed by IC_{50}…..**47**

Table X: Correlation between the antioxidant properties and phytochemicals of rosemary leaves extract…….…**49**

Table XI: Inhibitory activities of rosemary extract towards α-glucosidase and lipase……..…**50**

Table XII: Correlation between the antidiabetic activities and phytochemicals of rosemary extract……...**51**

Table XIII: Changes in physicochemical parameters of samples throughout storage….……**58**

Table XIV: Surface color parameters of fresh cheeses along the storage period……..…**61**

Table XV. Concentration of individual phenolic compounds (in mg/100g of cheese) of fresh cheeses along the storage period…….……..…..**64**

Table XVI: Effect of rosemary supplementation on hedonic appreciation of cheese samples…….……**67**

Table XVII. Proteins concentration in whey-rosemary…….……...…**76**

List of figures

Figure 1: Morphology and taxonomy of *Rosmarinus officinalis* L. ..……...…...**7**

Figure 2: Chemical structure of rosmarinic acid…….…..**10**

Figure 3: Chemical structure of apigenin (A) and luteolin (B)……..……**11**

Figure 4: Chemical structure of carnosol (A) and carnosic acid (B)……...…...**12**

Figure 5 Proposed reactions of a phenolic acid compound with amino side chains of polypeptides ……...…..**28**

Figure 6. Graphical abstract corresponding to the experimental design.....…….……**31**

Figure7: Microwave oven used for microwave-assisted extraction……..……**32**

Figure 8: Microwave-assisted extraction parameters effect on total phenolic yield from *Rosmarinus officinalis* L. leaves…….……...**44**

Figure 9: Chromatographic profile of the rosemary leaves extract with quantitative characterization of the prominent compounds…….……...**46**

Figure 10. Total phenolic content and antioxidant activity of cheeses as affected by rosemary addition during storage…….……**63**

Figure 11: Evolution of the lactic flora of cheese samples during storage…….……..**66**

Figure 12: Preference mapping of panelists…….……**68**

***Figure 13*:** Relationship between physicochemical parameters, sensory attributes phenolics and antioxidant capacity of cheese samples……**71**

Figure 14: Effect of rosemary extract concentrations on whey……..…...**76**

Figure 15: Variation in optical density depending on the concentration of *Rosmarinus officinalis* extract ……...**76**

Figure 16: Effect of varying the concentration of caffeic acid on protein complexation……...**77**

Figure17: Variation of the optical density as a function of the concentration of caffeic acid…**78**

General introduction

General introduction

Recently, several studies underlined a growing interest in developing functional foods with health promoting natural ingredients, especially of plant origin, for increasing consumer acceptability, palatability, stability and/or shelf-life of food products (Djaoud, Boulekbache-Makhlouf, Yahia, Mansouri, Mansouri, Madani, et al., 2020; Granato, Nunes, & Barba, 2017). These ingredients have started to replace the synthetic preservatives; extracts from spices, such as rosemary, could prove useful in preventing lipid oxidation and the development of off flavors and aromas (Ahmed S Gad & Sayd, 2015).

Labiatae (Lamiaceae) is an important family that consists of 250 genera and more than 7000 species. *Rosmarinus officinalis* L., belonging to this family is more commonly known as rosemary and is a popular spice originating from Mediterranean areas (de Raadt, Wirtz, Vos, & Verhagen, 2015; Linares, Arráez-Román, Herrero, Ibáñez, Segura-Carretero, & Fernández-Gutiérrez, 2011).

Rosemary has been shown to contain several important and high market value phenolic antioxidants such as phenolic diterpenes and phenolic acids (Birtić, Dussort, Pierre, Bily, & Roller, 2015; de Raadt, Wirtz, Vos, & Verhagen, 2015; Lemos, Lemos, Pacheco, Endringer, & Scherer, 2015; Loussouarn, Krieger-Liszkay, Svilar, Bily, Birtić, & Havaux, 2017). Several researchers have examined the phenolic profile of rosemary extracts, as well as their potential beneficial effects, mostly antioxidant and antimicrobial activities among others (Borrás-Linares et al., 2015; Borrás-Linares et al., 2014; Lemos et al., 2015; Tavassoli & Djomeh, 2011).

In particular fermented milk, cheese, yoghurt and ice cream are the best source of calcium and proteins, especially of caseins. These proteins are arranged in the form of micelles that are held together by hydrophobic interactions, hydrogen bonds and calcium phosphate nanoclusters, and whey proteins: the globular proteins as β-lactoglobulin, α-lactalbumin, immunoglobulins, and bovine serum albumin (BSA) (Yildirim-Elikoglu & Erdem, 2018). Cheese making involves the conversion of liquid milk into cheese via the activity of rennet enzyme with/without further

treatments of the curd, depending on the type of cheese (Yildirim-Elikoglu & Erdem, 2018). Cheese is a dairy product widely appreciated worldwide and its production and its consumption are continuously increasing.

In the literature, only a limited number of studies have focused on fermented milks fortified with rosemary. In this regard, Ahmed Saad Gad and El-Salam (2010) assessed the effect of rosemary aqueous extract added to skim milk through processing on phenolic compounds and antioxidant levels, as well as the effect on the rennet coagulation time of the milk. In addition, Hala, Ebtisam, Sanaa, Badran, Marwa, and Said (2010) prepared an UF-soft cheese with different percentages of rosemary extract having high phenolic content and antioxidant capacity. Furthermore, Gad et al. (2010) studied the production of functional yoghurt supplemented with rosemary extract as natural antioxidant. In fact, the aromatization of fermented milk with rosemary is well known in some specific areas of Bejaia (Algeria). This research is done in the concept of support and preservation of this culinary tradition by providing relevant scientific information needed to support this product and allow its manufacture at industrial scale. For that purpose, fresh cheese was supplemented with rosemary leaves (powder/phenolic extract) to evaluate the impact of this addition on the physicochemistry of milk and the phytochemistry of the plant.

The main objectives of each study are:

1) To determine the optimal conditions required for microwave-assisted extraction of antioxidants from rosemary leaves and study of biological activities and the phenolic composition of the extract obtained;

2) To investigate the effect of rosemary leaves addition to fresh cheese formulations on physicochemical parameters, antioxidant capacity and sensory characteristics of cheeses;

3) To test the ability of rosemary leaves as functional ingredients in fresh cheese, through monitoring, during refrigeration storage, its potential impact on microbiological quality

parameters, phenolic composition as well as the antioxidant capacity and comparison of results obtained with the plain fresh cheese.

The paper contains two parts, namely: the first part presents a literature study on the subject, while the second part deals with the original experimental part.

The literature study is further structured into two chapters. **Chapter I** presents the literature referring to the general aspects of the selected plant (rosemary). **Chapter II** presents a literature study on the fresh cheese.

According to the objectives, *Figure 1* schematically represents the experimental design of all the assays performed in the framework of this thesis.

The experimental part is divided into three chapters. In **Chapter III,** the research performed for the optimization of extraction of polyphenols from rosemary leaves is presented. This chapter describes the microwave assisted extraction (MAE) of polyphenols from rosemary, considering the influence of various parameters on the process. MAE of phenolic compounds from *Rosmarinus officinalis* L. was optimized using response surface methodology (RSM). A Box-Behnken Design (BBD), which is one of the most efficient designs of experiment methods, was adopted for the experiment planning. Furthermore, a phenolic characterization of the obtained extract, under the optimum conditions, was determined as well as some of its biological activities (antidiabetic and antioxidant effects). In **Chapter IV,** the research performed on the incorporation of rosemary leaves in fresh cheese formulations is presented. This chapter describes the study of the influence of this incorporation on some physicochemical characteristics, sensory attributes, the antioxidant capacity as well as the microbiological quality parameters along the storage period. **Chapter V** represents the main results obtained and proving the whey proteins-rosemary polyphenols complexation.

The general conclusion and perspectives that this work suggests were presented in the last chapter of this thesis.

Literature review

Chapter I:

General informations on

Rosemary

CHAPTER I – General informations on rosemary

I.1. The Labiatae family (Lamiaceae)

Lamiaceae (Labiateae) is one of the largest and most distinctive families of flowering plants which consist of 236 genera and almost 7173 species worldwide (Zahra & Shinwari, 2016). A large number of Lamiaceae species inhabit different ecosystems and have a great diversity with a cosmopolitan distribution. Hedge (1992) recognized six regions of high Lamiaceae diversity: Mediterranean and SW Central Asia; Africa south of the Sahel and Madagascar; China; Australia; South America; Northern America and Mexico.

Labiatae are known for their essential oils common to many members of the family. Many biologically active essential oils have been isolated from various members of this family. These plants have been used by humans since prehistoric times. The family is also famous for the presence of diterpenoids in its members. Most of the species are aromatic and possess a complex mixture of bioactive compounds (essential oils, diterpenoids …) that contribute to overall biological activity in both in vitro and in vivo conditions. Moreover, plants that belong to this family, such us species of *Mentha*, *Thymus*, *Salvia*, *Origanum*, *Coleus* and *Rosmarinus*, are valuables and useful in food, cosmetics, flavoring, flagrance, perfumery, pesticide, and pharmaceutical industries. Because of a wide range of applications, the plants of the Lamiaceae family are widely cultivated and are, therefore, regarded as an indispensable source of functional food (Milan Stankovic, 2020).

I.2. Brief history of rosemary

The word rosemary derived from the Latin word ros-rosis (dew) and marinus (sea), which means ‘dew of the sea’. It was also called ‘antos’ by the ancient Greeks, which is ‘the flower’, or ‘libanotis’ because of its incense smell (Begum et al., 2013; Pintore et al., 2002). In the past, nearly two thousand years ago, this aromatic plant was introduced in some European countries, like Britain, Greece and Italy, where rosemary was believed to invigorate the memory and was

remembered as the plant of fidelity and remembrance (Begum et al., 2013). It was also associated with love and marriage, once it is still used in bridal bouquets and in the crib of a new baby to protect him/her against bad influences and forces (Al-Sereitia, Abu-Amerb, & Sena, 1999). The plant was also used to recover speech, to enhance menstrual flow and stimulate hair growth, to help digestion and against flatulence and liver disorders (Heinrich, Kufer, Leonti, & Pardo-de-Santayana, 2006). In ancient China, rosemary was used for headaches, insomnia, and mental fatigue and topically for baldness (Leung and Foster, 1996). In India, rosemary enjoys a history of use as a stimulant and as a carminative to expel gas from the stomach and intestines and as antimigraine (Nadkarni, 1994). Anthropologists and archaeologists found evidence that this aromatic plant was used as culinary, medicinal and cosmetic in the ancient Egypt, China, India and Mesopotamia (Stefanovits-Banyai, Tulok, Hegedus, Renner, & Varga, 2003). This plant was cultivated worldwide since long ago and over the years rosemary was introduced as a garden plant, since its fragrance is quite pleasant (Begum et al., 2013; Heinrich et al., 2006; Moreno et al., 2012; Ulbricht et al., 2010).

I.3. Botanical description, distribution and cultivation

Rosmarinus officinalis L. is generally known as rosemary, common rosemary or garden rosemary (Begum et al., 2013; Ulbricht et al., 2010). It belongs to Lamiaceae (previous called Labiatae) family (Ali, Saleem, Ali, & Ahmad, 2000; Begum et al., 2013).

Rosemarinus officinalis is an evergreen perennial with fragrant shrub reaching up to 2 m height. Its leaves are strongly curved, leathery, linear and aromatic with prominent midrib, the upper surface of the leaf is dark green and the lowered surface is grey, with 1.0-2.5 cm long and 4 cm width by 1-3 mm thick (Begum et al., 2013; Lorenzi & Matos, 2006). The corolla is two-lipped and the stamens are curving outwards beyond the corolla (Arnold et al., 1997). Rosemary flowers are small and light blue or lilac (Arnold et al., 1997; Lorenzi & Matos, 2006). Leaves and flowers have a strong fragrant characteristic odour due the volatile oil accumulated in

typical peltate and capitate glandular trichomes (Díaz-Maroto, PerezeCoello, Sanchez-Palomo, & Gonz_alez Vi~nas, 2007; Marin et al., 2006).

Besides *Rosmarinus officinalis*, the genus also includes *Rosmarinus eriocalyx*, *Rosmarinus tomentosus*, *Rosmarinus lavandulaceus* and *Rosmarinus laxiflorus* (Angioni et al., 2004; Elamrani, Zrira, Benjilali, & Berrada, 2000; Rossello et al., 2006; Upson, 2006; Zaouali, Bouzaine, & Boussaid, 2010). *Rosmarinus officinalis* is the only that grows naturally in the Mediterranean region and the most exploited species due to its valuable essential oil (EO) but also due to its phenolic content and antioxidant activity (Zaouali et al., 2010).

More than 20 different types, varieties or cultivars of *Rosmarinus officinalis* can be distinguished according to morphological descriptors (like calyx, corolla, dimension of leaf, inflorescence and the presence of glandular trichomes) (Begum et al., 2013; Zaouali et al., 2010).

Rosemarinus officinalis is a xeromorphic species that grows spontaneously on sand, cliffs and stony places, near the sea, in different parts of the world (Arnold et al., 1997; Bakirel et al., 2008; Miraldi, Giachetti, Mazzoni, & Biagi, 2010). It is trusses of blue flowers last through spring and summer in a warm, humid environment. Plant comes up well in Mediterranean climate. It is indigenous to South Europe, Asia Minor and North Africa. It grows wild on Mediterranean shores and in Spain, Portugal, Morocco and Algeria and is cultivated in Spain, Tunisia, Yugoslavia, France, Italy, North Africa and India. Plant is susceptible to frost injury. In cooler areas, it can be cultivated in summer season. In a warm climate, plant remains in same location for up to 30 years, but in climates where freezing temperatures are expected, plants may be grown in pots so that it can be brought indoors in winter. Rosemary succeeds best in a light, rather dry soil. On a chalk soil it grows smaller, but is more fragrant (Barbara Dawson, 2020).

Taxonomie

Kingdom: Plantae
Subkingdom: Tracheobionta – Vascular plants

Superdision: Spermatophyta – Seed plants
Division: Magnoliophyta – Flowering plants

Class: Magnoliopsida – Dicotyledons
Subclass: Asteridae

Order: Lamiales
Family: Lamiaceae
Genus: *Rosmarinus*
Species: *Rosmarinus officinalis* L.

Figure 1: Morphology and taxonomy of *Rosmarinus officinalis* L. (Köhler, 1897, I.S.H.S., 1990)

I.4. Nutritional composition (macro and micronutrients)

The proximate and minerals content found in rosemary are shown in Tables I and II, respectively. The variation on macro and micronutrients content can be greatly attributed to the differences in species, varieties, growth conditions, harvesting times, soil properties, climate, origin, and geographic parameters (Arslan & Ozcan, 2008; Ozcan, Ünver, Uçar, & Arslan, 2008).

Nutrients, as well as compounds responsible for flavour and colour can be degraded due to the high temperatures and long times applied in some drying methods (Szumny et al., 2010).

Aromatic plants provide protein, fibre, volatile components (Essential oil), vitamins (A, C and B), minerals (Ca, P, Na, K and Fe) and chemical compounds that are known to have disease preventing and promoting health properties (USDA, 2015).

Aromatic plants are an excellent substitute of salt due to their composition and nutritional health functions. Excessive consumption of salt influences the appearance of hypertension, stomach cancer or stroke. The use of plants like rosemary is recommended by Portuguese authorities to help reduce added salt to food (Direç~ao-Geral da Saúde [DGS], 2014).

Table I: Nutritional value of rosemary plant

Component	Units	Fresh rosemary		Dried rosemary		
Energy	kcal	131	96	331	331	345
Water	g/100 g	67.77	73.6	9.31	9.3	9.3
Protein[a]	g/100 g	3.31	1.4	4.88	4.9	5.0
Total lipids (Fat)	g/100 g	5.86	4.4	15.22	15.2	15.2
Total carbohydrates	g/100 g	20.70	–	64.06	–	46.4
Total dietary fibre	g/100 g	14.10	7.7	42.6	17.7	24.1
Fatty acids						
Total saturated	g/100 g	2.84	1.14	7.371	3.94	–
Total monounsaturated	g/100 g	1.16	0.29	3.014	0.99	–
Total polyunsaturated	g/100 g	0.90	2.25	2.339	7.76	–
Vitamins						
Total ascorbic acid	mg/100 g	21.8	29	61.2	0.00	50
Thiamin	mg/100 g	0.036	0.10	0.514	0.10	0.5
Riboflavin	mg/100 g	0.152	0.21	0.428	0.32	0.00
Niacin	mg/100 g	0.912	1.0	1.00	1.0	1.00
Vitamin B_6	mg/100 g	0.336	0.09	1.740	0.38	0.00
Vitamin E	mg/100 g	–	1.50	–	9.15	0.00
Folate	µg/100 g	109	0.00	307	0.00	0.00
Vitamin B_{12}	µg/100 g	0.00	–	0.00	–	0.00
Vitamin A, RAE	µg/100 g	146	92.0	156	313	313
Vitamin D ($D_2 + D_3$)	µg/100 g	0.00	0.00	0.00	0.00	0.00
References		USDA	BDA	USDA	BDA	BEDCA

USDA = United States Department of Agriculture; BDA = Banca Dati di Composizione degli Alimenti per Studi Epidemiologici; BEDCA = Base de Datos Espa~nola de Composici_on de Alimentos.[a] Nitrogen Conversion Factor - 6.25.

However, there is limited information about aromatic plants nutritional composition. Some aromatic plants such as basil, cinnamon, cloves, coriander, cumin, fennel, ginger, nutmeg, oregano, parsley, rosemary, sage and thyme can be found in the American, Australian, Brazilian, Canadian, Danish, Finn, French, Italian, Japanese, Portuguese, Spanish, and Swedish food composition databases. Dumbrava, Moldovan, Raba, and Popa (2012) identified the vitamin C content of raw materials (18.51 g/100 g), aqueous (0.26 mg/100 mL), alcoholic (0.34 mg/100 mL) and acetonic (0.36 mg/100 mL) extract of rosemary leaves.

The authors also quantified chlorophyll "a" (14.1 mg/L) and carotenes plus xanthophylls (30.6 mg/L) in alcohol leaves extract. On the other hand, Loranty, Rembiałkowska, Rosa, and Bennett (2010) did not detect any pigments in the infusion of rosemary.

Table II: Minerals content (mg/100 g) of rosemary

Minerals (mg/100 g)	Plant part								
	Dried leaves					Fresh leaves		Aerial	
	–	–	Oven	Sun	Microwave oven	Fresh	Fresh	Dried	Infusion
Ag	–	–	–	–	–	–	–	0.01	0.00
Al	115	–	24.0	23.1	22.5	14.8	–	3.1	0.00
As	–	–	–	–	–	–	–	0.0	0.00
B	2.1	–	3.1	4.6	3.1	2.9	–	0.7	0.01
Ba	–	–	3.3	4.1	3.9	2.4	–	0.2	0.00
Bi	–		–	–	–	–	–	0.02	0.00
Ca	860.5	1280	1358.7	1234.3	1360.0	779.2	317	22.4	2.5
Cd	0.07	–	–	–	–	–	–	0.005	0.00
Co	–	–	0.2	0.2	0.1	0.1	–	0.04	0.00
Cr	1.9	–	1.7	15.3	11.9	9.7	–	–	0.00
Cu	0.3	–	0.5	0.5	0.5	0.4	–	–	0.00
Fe	73.4	29.2	76.0	53.5	50.7	33	6.6	–	0.01
K	1111.6	955	2110.4	1961.2	1614.9	1491.6	668	–	9.0
Li	0.3	–	0.7	0.6	0.5	0.4	–	–	0.01
Mg	240.8	220	280.7	261.8	169.8	163.4	91	–	1.0
Mn	2.8	–	3.2	2.9	2.1	1.5	–	0.2	0.01
Na	489.4	50	444.8	437.7	297.7	271.2	26	4.34	0.01
Ni	0.8	–	6.0	4.7	4.16	1.9	–	0.04	0.00
P	820.5	70	412.2	210.6	289.5	107.5	66	146.6	0.01
Pb	0.2	–	–	–	–	–	–	0.034	0.00
Se	–	–	–	–	–	–	–	0.4	0.00
Sr	6.7	–	11.5	10.4	8.8	7.5	–	0.2	0.00
Ti	–	–	–	–	–	–	–	0.7	0.00
V	0.99	–	0.1	0.09	0,07	0.04	–	0.006	0.00
Zn	3.1	3.2	4.9	4.2	2.8	2.3	0.9	0.7	0.00
Reference	Özcan & Akbulut, 2007	USDA	Arslan & Özcan, 2008	Arslan & Özcan, 2008	Arslan & Özcan, 2008	Arslan & Özcan, 2008	USDA	Özcan et al., 2008	Özcan et al., 2008

I.5. Rosemary secondary metabolites

Plants produce large, diverse array of organic compounds that appear to have no direct function in plant growth and development. These substances are known as secondary metabolites, secondary products, or natural products (Hartmann, 1996), while primary metabolites (proteins, carbohydrates and fats) are important in plant physiological process such as growth and development (Mann, 1987). Secondary products have a variety of functions in plants.

It is likely that their ecological function may have some potential medicinal effects for human. For example, secondary products involved in plant defense through cytotoxicity toward

microbial pathogens could prove useful as antimicrobial medicines in human, if not too toxic (Briskin, 2000).

I.5.1 Phenolic compounds

The term phenolic compound includes a wide range of plant substances which possess in common an aromatic ring bearing one or more hydroxyl substituent. Some phenolic substances tend to be water-soluble, some are soluble only in organic solvents and others are large insoluble.

Phenolic compounds are formed via the shikimic acid pathway or malonic acid pathway (Taiz and Zeiger 2002). These phenolic metabolites function to protect the plants against biological and environmental stresses and therefore are synthesized in response to pathogenic attack such as fungal or bacterial infection or high energy radiation exposure such as prolonged UV exposure (Shetty, 1997 and Briskin, 2000).

a. Phenolic acids

Rosemary leaf contains 2-3% phenolic acids such as caffeic, chlorogenic, labiatic, neoclorogenic and rosmarinic acid (Newall *et al.*, 1996). Rosmarinic acid (Fig.2) the diphenolic compound is an ester of caffeic acid and 3,4-dihydroxyphenyl lactic acid. It is commonly found in species of Lamiaceae family. Rosmarinic acid is regarded as a potential pharmaceutical plant product and is noted for its potent antioxidant properties (Exarchou *et al.*, 2000; Malencic *et al.*, 2000; Ly *et al*., 2006).

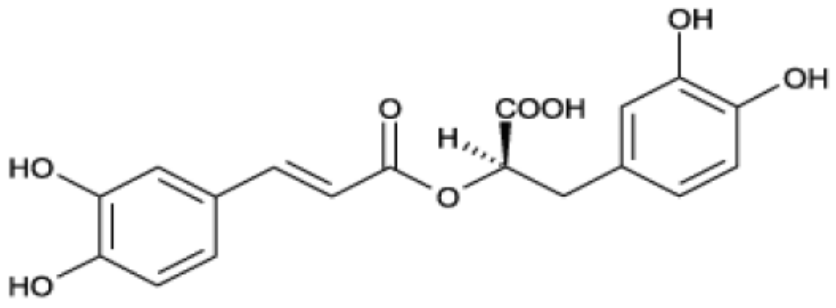


Figure 2: Chemical structure of rosmarinic acid (Almela *et al.*, 2006).

b. Flavonoids

Flavinoids in rosemary include diosmetin, diosmin, genkwanin, eriocitrin, hesperidin and hispidulin and two common flavones apigenin and luteolin (Fig.3) (Del Bano *et al.*, 2004; Almela *et al.*, 2006). Three flavonoids including glucuronides, luteolin 3'-O-beta-D-glucuronide and hesperidin, were isolated from 50% methanol extract of the leaves of rosemary.

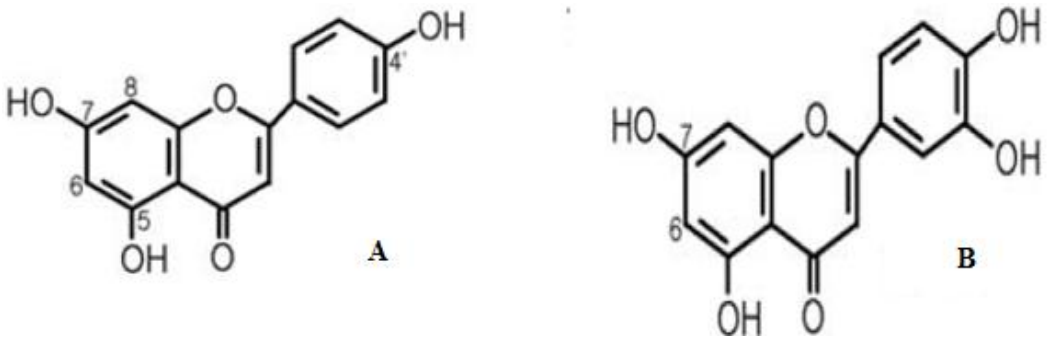


Figure 3: Chemical structure of apigenin (A) and luteolin (B) (Almela *et al.*, 2006).

c. Terpenoid compounds

Terpenoids are aromatic chemical compounds which are dissolved in fats; it's found in the cytoplasm of plant cells or in special glands like the volatile oil, or it could be found in green plastids (Harborne, 1993).

Terpenoids are based on the isoprene molecule and built from the union of two or more of (C5) units.

Terpenoids are classified according to whether they contain two (C10) monoterpenes, three (C15) sesquiterpenes, four (C20) diterpenes, six (C30) triterpenes or eight (C40) carotenoids units (Trease and Evans, 1989).

- **Diterpenes**

The most effective antioxidants in rosemary is the tricyclic diterpens carnosic acid in concentrations higher than 4% (Fig.4). Some other less effective diterpens in this plant are degradation products of carnosic acid, which are mostly converted to carnosol, carnosic acid

methylester, epirosmanol, rosmanol and 7-methylrosmanol (Schwarz and Ternes, 1992; Okamura *et al.*, 1994a; Cuvelier *et al.*, 1996).

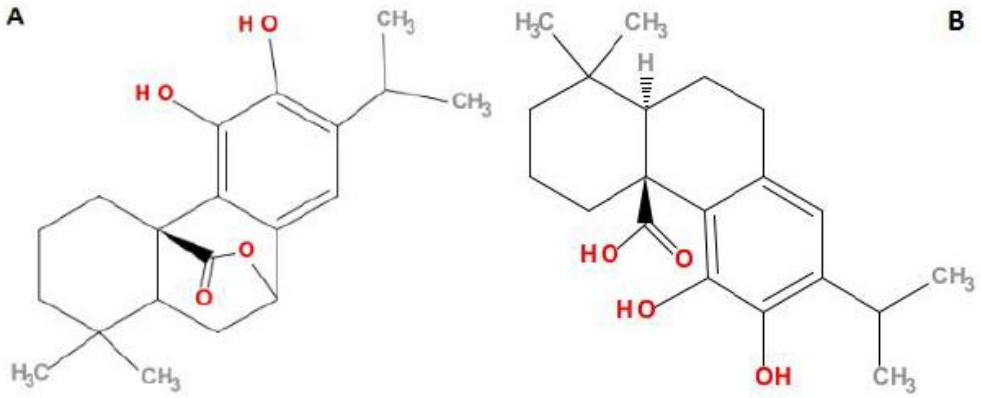


Figure 4: Chemical structure of carnosol (A) and carnosic acid (B) (Senorans et *al.*,2000)

Diterpenes are not found in all rosemary tissues. The leaf was the tissue showing the highest concentrations of diterpenes. Diterpenes are also present in the flowers of rosemary, although the concentrations found in the sepals are not comparable with those found in the petals. Sepals contained approximately 30% fewer diterpenes than leaves, but 3.2 times more than petals. Diterpenes were also found at low concentrations in seeds and trace amounts were detected in stems. Roots do not contain diterpenes (Munne-Bosch and Alegre, 2001).

- **Triterpenes**

Other terpenoid constituents in rosemary include triterpenes oleanolic and ursolic acids at 2-4% (Newall *et al.*, 1996).

I.5.2. Essential oils

The leaves of rosemary contain between 1-2.5% essential oil, such composition may markedly vary according to the chemo type and the development stage at which the plant has been harvested. It is an almost colorless to pale yellow liquid with a characteristic, refreshing and pleasant odor (Bauer *et al.*, 1997). Twenty components were identified in rosemary oils. The main constituents are α-pinene (40.55-45.55)%, 1,8-cineole (17.40-19.35)%, camphene (4.73-

6.06)%, verbenone (2.32-3.86)%, borneol (2.24-3.10)%, bornyl acetate, α-terpineol camphor, limonene, β-pinene and geraniol <2% (Atti-Santos *et al.*, 2005).

I.5.3 Alkaloids

Alkaloids are a large group of secondary products offer protection against predators, act as growth regulators, maintain ionic balance, act as a nitrogen reserve and possibly serve as nitrogen excretion products and exhibit important pharmacological properties. They are substances containing one or more nitrogen atoms in combination as part of a cyclic system. The most common precursors of alkaloids are amino acids (Goodwin and Mercer, 1983).The nitrogen-containing secondary products are biosynthesized primarily from amino acids (Taiz and Zeiger, 2002). Water extract of rosemary contain the alkaloid rosmaricine7 (Hoefler *et al.*, 1987).

I.6. Rosemary biological activities

I.6.1. Antimicrobial activity

Different extracts of rosemary have demonstrated inhibitory activity on cultures of *S. aureus*, *S. albus*, *E. coli*, *Corynebacterium spp*., *Bacillus subtilis*, *Micrococcus luteus*, *Salmonella spp*., *Listeria monocytogens* and *Vibrio cholerae*.

Two of its components, carnosol and ursolic acid, are responsible for this antimicrobial effect and fungi of the *Aspergillus sp*, *Penicillium spp.*, *Alternaria spp.* genera and other food fermenting microorganisms such as *Lactobacillus brevis*, *Pseudomonas fluorescens*, *Rhodotorularia glutinis* and *Kluyveromyces bulgaricus* (Alonso, 2004).

Methanol extract is containing 30% of carnosic acid, 16% of carnosol and 5% of rosemarinic acid. It is an effective antimicrobial against Gram-positive bacteria, Gram-negative bacteria and yeast (Moreno *et al*., 2006).

Rosemary essential oil, like many essential oils, has antimicrobial properties when it comes in direct contact with bacteria and other microorganisms (Oluwatuyi *et al.*, 2004; Santoyo *et al.*, 2005).

Alpha-Pinene, beta-Pinene, 1,8-cineole, camphor, verbenone, and borneol standards showed antimicrobial activity against all the microorganisms tested, borneol being the most effective followed by camphor and verbenone Gram-positive bacteria (*S. aureus* and *B. subtilis*), Gramnegative bacteria (*E. coli* and *P. aeruginosa*), a yeast (*C. albicans*), and a fungus (*Aspergillus niger*), therefore, the active ingredients with bactericidal activity from rosemary extract are ideal when formulating purifying and antiseptic cosmetic products (Santoyo *et al.*, 2005).

One of the most recent studies to look at the effect of rosemary essential oil on drug-resistant infections found rosemary effective against several of the most common pathogens affecting humans, including the fungi that cause nail infections and the pathogen responsible for most vaginal yeast infections (Luqman *et al.*, 2007).

I.6.2. Antioxidant activity

As one of the most potent antioxidants known, rosemary prevents free radical damage, protects cells from deterioration and aids in the prevention of cancer.

Research into the free-radical quenching effects of rosemary has found it to be a potent antioxidant, possessing greater activity than the common food additives. The discovery of the antioxidant activity of rosemary in biological systems supports the historical use of rosemary as a preservative for meats and foods (Ho *et al.*, 1994).

I.6.3. Anti-inflammatory activity

Rosmarinic acid is a naturally occurring non-steroidal anti-inflammatory agent. It inhibits the complement factor C3, a mediator in the inflammatory process (Al-Sereitia *et al.*, 1999; Alonso,

2004). Rosmarinic acid has a scavenging effect on the active oxygen free radical (Zhao *et al.*, 2001).

Manez *et al.* (1997) verified that ursolic acid reduces the chronic inflammation and neutrophil infiltration. This activity is closely linked to the structure of this compound. Rosemary extract is highly recommended when formulating cosmetic products for sensitive or irritated skin.

I.6.4. Antiseptic activity

As an antiseptic, rosemary cleanses the blood and helps to control many pathogenic organisms. It is potent enough to kill bacterial infection but not so potent, however, to completely wipe out the natural bacterial populations of the digestive tract that keep the intestines in healthy balance. Rosemary's antiseptic quality heals wounds (Williamson *et al*., 1988; ESCOP, 2003).

Distilled water from the flowers is used as eyewash (Chiej, 1984). It can be used as a disinfectant, as a mouth wash and to treat fever or rheumatism (Calabrese *et al.*, 2002).

I.6.5. Rosemary medicinal uses

One of the potential therapeutic effects of rosemary is its role in preventing cancer. Researchers have demonstrated that natural polyphenols found in rosemary have potent anticarcinogenic properties. To date, rosemary extract, or its active components, carnosol, carnosic acid, and rosmarinic acid, have been shown to prevent cancer (Singletary *et al.*, 1996; Zhu *et al.*, 1998). Scientists have found that rosemary extract can significantly help to protect DNA against free radical damage (Slamenova *et al.*, 2002).

Rosemary is an excellent memory and brain stimulant that is said to improve brain function by feeding it with oxygen-rich blood and it play a role in memory loss associated with Alzheimer (Duke, 1997). It helps with headaches, migraines, neuralgia, mental fatigue and nervous disorders (Amini, 1997). Also rosemary is a central nervous system stimulant (Kovar *et al.*,

1987). Carnosic acid and carnosol are able to promote markedly enhanced synthesis of nerve growth factor (Kosaka and Yokoi, 2003).

Rosemary infusion used as a mouthwash alleviates halitosis (Chevallier, 1996). It is used internally for flatulence, stimulating the digestion, dyspepsia, carminative and as appetizer (Amini, 1997; Blumenthal *et al.*, 2000; ESCOP 2003; Mills and Bone 2005; Bradley, 2006; Barnes *et al*., 2007). Rosemary is used as an antispasmodic in renal colic, relaxes smooth muscles of intestine and its constituents have a therapeutic potential in treatment or prevention of liver damage and peptic ulcer (Al-Sereitia *et al.,* 1999; Masuda *et al.,* 2002; Sotelo-Fleix *et al.,* 2002; Osakabe *et al.,* 2004). It improve hepatic and biliary function and increases bile flow (Hoefler *et al*., 1987; ESCOP, 1997).

Rosemary improves blood circulation and decreases the permeability and fragility of the capillaries due to the flavinoid (diosmin) (Bown, 1995).

The plant has significant antithrombotic activity (blood thinning) and lowers blood pressure in hypertension patients (Yamanoto *et al.*, 2005).

Rosemary has therapeutic properties as a mild diuretic making it effective in reducing swollen ankles and bloating. Its diuretic action increases the flow of urine that flushes bacteria from the body before they have chance to cause infection (Haloui *et al.*, 2000).

Rosemary and its constituents have a therapeutic potential in treatment or prevention of bronchial asthma and in relieving respiratory disorders (Al-Sereitia *et al.,* 1999; Masuda *et al.,* 2002; Sotelo-Fleix *et al.,* 2002; Osakabe *et al.,* 2004). It is effective for asthma, bronchitis, catarrh, sinus, whooping cough, colds, cough and influenza (Small, 1997). Constituents of this herb include eucalyptol relaxes smooth muscles of trachea and lungs (Aqel, 1991).

Rosemary oleanolic acid extract decreased pain in patients suffering from rheumatic conditions and arthritis (Lukaczer *et al.,* 2005). Rosemary facilitates menstruation and regulates its flow, treating low or excessive bleeding and its constituents have a therapeutic potential in treatment of poor sperm motility, rosemary and its constituents have a therapeutic potential in treatment

or prevention of cataract (Al-Sereitia *et al.,* 1999; Masuda *et al*., 2002; Sotelo-Fleix *et al*., 2002; Osakabe *et al*., 2004).

Rosemary enhances immunity because it stimulates sweating and toxin removal (Babu *et al*., 1999). It alleviates allergies due to the ursolic acid content of rosemary inhibiting mast cell degranulation and histamine release from mast cells in the presence of antigens (Tsuruga *et al.,* 1991). It exhibited strong inhibitory effects against human immunodeficiency virus (HIV) (Paris *et al.*, 1993).

Ursolic acid and oleanolic acid, have been used in tonics to enhance hair growth and prevent scalp irritation, they encourage hair growth by stimulating the peripheral blood flow in the scalp and activating the hair mother cells. They also furnish alopecia-preventing and dandruff-preventing effects (Okazaki *et al.*, 1989; Kikuko *et al.*, 1993).

I.7. Uses of rosemary extracts in foods

I.7.1. Function in foods

Unless intentionally removed, the extracts of rosemary still contain flavourings and can be used to improve the flavour of products; however rosemary extracts are currently widely used to increase shelf life of food products due to the high antioxidant activity of its main components (Almela et *al*., 2006, Hernández-Hernández et *al*., 2009**)**. Research on the antioxidant activity of the compounds in rosemary extract in foods is primarily focused on carnosic acid. The potency of carnosic acid in comparison to the other compounds in rosemary extracts in soybean oil was found to be more than twice as high based on Rancimat measurements (Richheimer et *al*., 1996). Furthermore, the same study also found higher antioxidant activity for carnosic acid in comparison to the synthetic antioxidants BHT and BHA, whereas tertiary butylhydroquinone (TBHQ) was found to surpass rosemary extracts in antioxidant activity. Zhang et al. (2010) found the similar results when comparing BHT, BHA, and TBHQ with carnosic acid based on peroxide values, TBARS assays, free fatty acid measurements, and p-anisidine values in

sunflower oil. However, antioxidant activity of rosemary extracts seems to depend on the medium. Frankel et al. (1996) measured the antioxidant activity of both carnosol and carnosic acid in a corn oil emulsion and bulk oil using hydroperoxide and hexanal formations and found reduced antioxidant activity in the emulsion system. Furthermore, antioxidant activity at pH = 7 was lower compared to activity at pH = 4 and 5. Koleva et al. (2002) found a similar difference between bulk oil measurements and emulsion oil measurements for rosemaric acid.

The findings suggest that rosemary extracts might not increase shelf life in all products, but evidence exist for several food categories. Using the TBARS assay, Sebranek et al. (2005) found similar activity for an unspecified extracts as BHA and BHT in precooked frozen pork sausages, but in raw frozen pork sausages this extracts was found to be more effective than BHA and BHT in preventing increased TBARS values, and in preventing loss of red colour. Moreover, Nassu et al. (2003) investigated the efficacy of a commercial albeit unspecified rosemary extract in fermented goat sausage using a TBARS assay and found a significant difference in oxidation with 0.050% w/w extract compared to controls. Using peroxide values according to the AOCS (1989) method, Frutos et al. (2005) found significant protection against oxidation in bread with an oil, garlic and parsley dressing when using a 4 g/l concentration of rosemary extracts [carnosic acid (20–30%), rosmarinic acid (0–1%) and rosmanol (0.5–1.5%)]. Despite the possible differences in the extract composition, these studies depict the efficacy of rosemary extracts in prolonging oxidative degradation in actual food products, however no evidence was found for the approved rosemary extracts.

I.7.2. Dietary Exposure

In 2008 EFSA published its opinion on the use of extracts of rosemary as a food additive (Aguilaret al., 2008). As part of this opinion, expected dietary exposure was assessed based on consumption in the UK. The UK National Dietary and Nutrition Survey for adults (Henderson et al., 2002) and pre-school children (Gregory JR, et al., 1995) were used as the main data

sources. Despite the exclusion of rosemary as flavouring agent, a conservative estimate, based on the assumption of maximum usage levels in all proposed foods, was made. As reported by Paul de Raadt et al. (2015), the potential mean exposure to carnosol plus carnosic acid from all proposed uses was estimated at 0.04 mg/kg bw/day for adults and 0.11 mg/kg bw/day for pre-school children. At the 90^{th}, 95^{th} and $97,5^{th}$ percentile the expected intake was estimated at 0.08, 0.10 and 0.12 mg/kg bw/day for adults. For pre-school children this was estimated at 0.18, 0.20 and 0.23 mg/kg bw/day respectively.

Chapte II:

General information on fresh cheese and polyphenols-proteins interactions

CHAPTER II - General information on fresh cheese and polyphenols-proteins interactions

II.1. Cheese production and consumption

Cheese is the generic name for a group of fermented milk-base food products, made in a wide range of flavours and forms throughout the world. Traditionally, it was elaborated as a way of preserving milk and increasing its economic and nutritional values. Cheese is an excellent source of protein, fat and minerals such as calcium, iron and phosphorus, vitamins and essential amino acids and consequently is an important food in the human diet. However, its composition greatly depends on milk composition and the manufacture process.it is well known that some other factors such as climate, season, animal feed, age and breed, stage of lactation and health state of the animal, could influenced the composition of milk (Farkye, 2004).

It is estimated that there are in excess of 1000 distinct cheese varieties currently produced worldwide. With regard to cheese production and consumption aver the world, the last report of the Dairy Industries International (DII, 2019), the US (6.1 million tonnes) remains the largest cheese consuming country worldwide, accounting for 24% of total volume. Moreover, cheese consumption in the US exceeded the figures recorded by the second-largest consumer, Germany (3 million tonnes), twofold. The third position in this ranking was occupied by France (1.6 million tonnes), with a 6.4% share. From 2013 to 2019, the average annual rate of growth in terms of volume in the US totaled 2.5%. In the other countries, the average annual rates were as follows: Germany (+4.4% per year) and France (+1.3% per year). The countries with the highest levels of cheese per capita consumption in 2019 were the Czech Republic (64 kg per person), Germany (37 kg per person) and France (25 kg per person). From 2013 to 2019, the most notable rate of growth in terms of cheese per capita consumption, amongst the main consuming countries, was attained by the Czech Republic, while cheese per capita consumption for the other global leaders experienced more modest paces of growth.

In 2019, the amount of cheese produced worldwide rose slightly to 26 million tonnes, picking up by 2.6% on the year before. The total output volume increased at an average annual rate of 2.6% from 2013 to 2019; the trend pattern remained consistent, with somewhat noticeable fluctuations in certain years. The countries with the highest volumes of cheese production in 2019 were the US (6.3 million tonnes), Germany (3.5 million tonnes) and France (1.9 million tonnes), with a combined 46% share of global production. These countries were followed by Italy, Poland, the Netherlands, Argentina, Russia, the Czech Republic, Egypt, the UK and Canada, which together accounted for a further 26%.

According to the study conducted by Ramdane Sidali et al. (2019), milk and derivatives have a big place on the table of Algerians, with a national average annual consumption of 96 kg /person and 0.23 kg /person for cheeses.

II.2. Description of cheese

Cheese is described by *Codex Alimentarius* (CODEX-STAN-283-4, 2013) as being the "ripened or unripened, soft or semi-hard, hard and extra hard product", which may be coated; and in which the whey protein/casein ratio does not exceed that of milk obtained by:

- Coagulating wholly or partly the milk protein, skimmed milk or products, partly skimmed milk, cream, whey cream or buttermilk, through the action of rennet or other suitable coagulating agents, and by partially draining the whey resulting from such coagulation;
- Processing techniques involving the coagulation of the protein of milk and/or products obtained from milk, which gives a product with physical, chemical and organoleptic characteristics similar to the product defined. Include unripened cheeses (fresh cheeses) which are ready for consumption shortly after manufacture.

II.2.1. Coagulation of milk

The ability of casein micelles to stay in solution at natural milk pH (~6.7) relies on the net negative charge and hydrophilic character of the C-terminal end of κ-CN at the micelle surface.

There are two approaches to induce micelle aggregation; by enzymatic action (cheese) or by acidification (fermented milk products). The outcome of these reactions is to a large extent determined by amounts and proportions of the various components in milk, with the protein composition contributing significantly in this regard. To determine the coagulation properties of given milk, different traits to describe the process are measured. Coagulation time (CT), defined as the time from addition of coagulant until coagulation starts, and curd firmness at a given time after addition of coagulant.

- **Enzyme-induced coagulation**

Enzymatic coagulation of milk is the modification of casein micelles via limited hydrolysis of casein by rennet, followed by calcium-induced micelle aggregation (Fox & McSweeney, 2004). Rennet is traditionally extracted from calf abomasa and is a mixture of the two gastric proteases chymosin and pepsin (Andrén, 2002). Chymosin is the major and the most active component, specifically cleaving the peptide bond Phe105-Met106 of κ-CN. Chymosin-induced coagulation of milk may be described by three phases. During the primary phase the enzymatic hydrolysis of κ-CN into para-κ-CN and CMP takes place, with the hydrophilic CMP part being released into the whey. This causes loss of a negatively charged group and decreased steric stabilisation (Senge *et al.*, 1997). When approximately 70 % of the κ-CN is hydrolysed (Walstra *et al.*, 2006), colloidal stability of the micelles is reduced enough for the spontaneous, secondary aggregation phase to start. A gel forms as molecular chains connect through hydrophobic bonds to form a three-dimensional network, followed by further solidification through calcium cross-linking. Finally in the third phase whey is expelled from the casein network by syneresis (more contraction through cross-links).

Coagulation is enhanced by decreasing pH, increasing calcium concentration and temperature (no aggregation below 20°C). Syneresis is augmented by increasing temperature, pH and applied pressure, e.g. stirring.

- **Acid-induced coagulation**

At acid coagulation of milk, casein micelle properties are altered by a lowered milk pH (Lucey & Singh, 1997). This causes CCP to dissociate from the micelles and the negative charges in the casein micelles are neutralized, with aggregation occurring as the isoelectric point of the casein micelle (pH 4.6) is approached. A porous network of loosely linked aggregates is formed. Milk used in manufacture of fermented milk products is generally subjected to a quite severe heat treatment (90°C, 5-10 min), with a marked effect on the end product. Temperatures above 60°C cause denaturation of whey proteins (mainly β-LG), which via disulphide bonds either associate with κ-CN on the casein micelles (McKenzie *et al.*, 1971; Sawyer, 1969) or form soluble aggregates (Guyomarc'h *et al.*, 2003a; Haque & Kinsella, 1988). This results in increased curd firmess (Dannenberg & Kessler, 1988a) due to an increased number and strength of bonds of the acid gel, as denatured whey proteins associated with casein micelles interact with each other (Lucey & Singh, 1997). Further, the concentration of protein in the gel network will be increased because of the active participation of denatured whey protein in structure formation.

- **Factors influencing coagulation of milk**

Coagulation of milk is a complex process, influenced by many different factors. The most obvious are pH, calcium content and temperature. Decreasing the pH and increasing the temperature will decrease the coagulation time. Regarding calcium, the coagulation reaction is favored both by increased levels of bound calcium (CCP) and free calcium ions. Adding calcium to the milk will increase these levels in addition to lowering pH.

Many factors are intertwined and the milk protein fraction, which has different effects on the coagulation properties and will be discussed more below, can vary with the presence of different genetic variants, but there are also effects of breed (Auldist *et al.*, 2004; Chiofalo *et al.*, 2000; Malossini *et al.*, 1996), stage of lactation (Ostersen *et al.*, 1997), parity (Lindström *et al.*, 1984; Schaar, 1984), season and feeding (Christian *et al.*, 1995; Macheboeuf *et al.*, 1993), and cow

health (Grandison & Ford, 1986). Milk from cows with mastitis is associated with a high pH and low levels of casein (Barbano *et al.*, 1991; Larsen *et al.*, 2004; Urech *et al.*, 1999) and has been suggested to have negative effects e.g. for the manufacture of cheese (Barbano *et al.*, 1991; Leitner *et al.*, 2006).

Milk that does not coagulate in the presence of chymosin has puzzled researchers at least since the 1920's (Cassandro *et al.*, 2008; Claesson, 1965; Comin *et al.*, 2008; Ikonen *et al.*, 1999a; Ikonen *et al.*, 2004; Jõudu *et al.*, 2007; Koestler, 1925; Losi *et al.*, 1982; Okigbo *et al.*, 1985c; Tervala & Antila, 1985; Wedholm *et al.*, 2006b). The causes of non-coagulating (NC) milk are not fully understood, largely due to the still elusive structure of the casein micelle and the complexity of the milk coagulation process with its numerous controlling factors. However, it has been recognized that in addition to NC milk that can be explained by cows being in very late lactation (Flüeler, 1978; Okigbo *et al.*, 1985c) or having mastitis (Koestler, 1925; Okigbo *et al.*, 1985a), the phenomenon cannot be fully explained by environmental factors as it is prevalent also in healthy cows in mid lactation (Ikonen *et al.*, 2004; Tyrisevä *et al.*, 2003). Ikonen *et al.* (1999a) observed large differences between breeding bulls in their proportion of daughters producing NC milk and suggested that the underlying cause was partly genetic. Recently, two candidate genes associated with NC milk were identified (Tyrisevä *et al.*, 2008). A genetic disposition to produce NC milk does not exclude significant influences of environmental factors. It has been shown that addition of calcium will restore coagulation of NC milk (van Hooydonk *et al.*, 1986), although not to the level of well coagulating milk (Okigbo *et al.*, 1985b).

II.2.2. Starter-free fresh cheese

Fresh cheese is the cheese which is ready for consumption shortly after manufacture. Fresh cheese is a soft cheese that is manufactured with little or no starter culture and subsequently

consumed within 3-7 days of manufacture, although the labeled expiring date is usually of 14 days. This variety is typically white, have relatively high moisture (46-57%) and high pH (>6.1), contains about 8-29% fat, 7-20% protein and 1-3% salt (Hwang & Gunasekaran, 2001), it has a crumbly texture resulting from fine milling before salting (Van Hekken & Farkye, 2003).

Table III : Nutritional value of fresh cheese (Richonnet, 2015)

Composition	Nutritional value
Moisture (%)	79
Energy (kcal/100g)	118
Carbohydrates (g/100g)	4
Fat (g/100g)	17
Saturate fatty acids (g/100g)	12
Proteins (g/100g)	9
Sodium (mg/100g)	520
Calcium (mg/100g)	95
Phosphorus (mg/100g)	140

There is a substantial market for fresh cheese due to the demands for new cheese flavours, varieties and products, the nutritional benefits such as its high mineral and protein contents, as well as, the economic advantages due to its relatively short or no ripening time and high yield.

II.2.3. Fresh cheese manufacturing

Milk for fresh cheese-making is generally standardized for fat content pasteurized at 70-80°C for 15-40 s. Once pasteurized, is adjust to 30-37°C and calcium chloride, rennet and salt are added. Sodium chloride is usually added directly to milk before coagulation for better homogeneity of salt content within the final product.

Curd salting contributes to decrease water activity (a_w), control enzymatic activity and microorganism growth, affect biochemical and physic-chemical changes and enhance the flavour of cheese (Guinee & Fox, 2004). Coagulation time ranges from 15-45 min depending

on the characteristics of the rennet used. The rennet-set curd is cut and cooked to desired firmness, and the whey is drained. When curd is drained, fat and casein are concentrated between 6-12 fold times (Fox & McSweeney, 2004). The curd is then finely milled and packed into moulds of different shapes and sizes.

II.2.4. Fresh cheese types

According to Luquet and Corrieu, (2005), unripened cheeses, with various characteristics organoleptic (plain, with fruit, flavored) include:

- White cheese beaten (smooth texture) or "country-style" cheeses. The denomination "white cheese" is reserved for unripened cheese which, when fermented, has not undergone other fermentations than lactic fermentation;
- The natural “little Swiss or Gervaise”; fresh pulped cheeses;
- The “Slightly salted cheese”, often flavored (garlic, herbs, pepper, etc.).

II.3. Polyphenol-Protein Interactions

Phenolic compounds, as the major secondary metabolites in plant foods, comprise more than 8000 identified substances (Guo et al., 2009). They have a common structural feature, an aromatic ring bonded to at least one hydroxyl group (Kroll et al., 2003), and are classified according to the number of carbon atoms in their structure. Flavonoids, phenolic acids, and less common stilbenes and lignans, are phenolic compounds occurring in plants (Crozier et al., 2009; Czubinski and Dwiecki, 2017).

Phenolic compounds in food undergo chemical changes during food processing and can interact with protein macromolecules during food production and processing, and even after food consumption (i.e. during digestion in the gastrointestinal tract) (Buitimea-Cantúa et al., 2017; Le Bourvellec and Renard, 2012; Parada and Aguilera, 2007). The molecular weight and

structural flexibility of a phenolic compound, as well as the number of hydroxyl groups and type of side chain have been identified as the key factors that affect phenolic–protein interactions (Buitimea-Cantúa et al., 2017; Czubinski and Dwiecki, 2017). Phenolic compounds with a higher molecular weight and a higher abundance of hydroxyl groups (providing more than one site for interaction) are thought to have a higher affinity for protein (Dubeau et al., 2010; Frazier et al., 2010; Jakobek, 2015; Xiao et al., 2011). Phenolic-protein interactions can be grouped into non-covalent interactions (reversible) and covalent interactions (mostly irreversible) (Prigent, 2005; Rawel and Rohn, 2010; Ozdal et al., 2013). Five types of non-covalent interactions were reported, including hydrogen bonds, electrostatic interactions, hydrophobic interactions, van der Waals interactions, and p bonds (Ali, 2013; Frazier et al., 2010; McRae and Kennedy, 2011; Poncet-Legrand et al., 2006; Prigent, 2005; Rawel and Rohn, 2010; Siebert, 2006). Among these, hydrophobic interactions and hydrogen bonds are the primary non-covalent forces that mediate phenolic–protein interactions. Amino acids, including alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, tryptophan, cysteine and glycine, may interact with phenolic compounds through hydrophobic interactions. Moreover, phenolic compounds are excellent hydrogen donors that form hydrogen bonds with the carboxyl group of proteins (Jongberg et al., 2015; Mulaudzi et al., 2012; Xiao et al., 2011) Consequently, hydrogen bonds may take place between the nitrogen or oxygen molecule of amino acids (e.g., lysine, arginine, histidine, asparagine, glutamine, serine, threonine, aspartic acid, glutamic acid, tyrosine, cysteine and tryptophan) and the hydroxyl groups of phenolics (Ali, 2013; Prigent, 2005; Rawel and Rohn, 2010).

Interestingly, the ability of phenolic compound to produce a quinone radical mediates the formation of a covalent bond (Jongberg et al., 2015). Under alkaline conditions and in the presence of oxygen, the phenolic compounds are susceptible to enzymatic and non-enzymatic oxidation reactions causing the generation of highly reactive quinone radicals. As the second step of oxidation, the quinone undergoes condensation reactions (reacting with another

quinone) to form a dimer, a high molecular weight brown colored pigment named as tannin, which can further react with amino acids in a polypeptide chain through covalent bonding (Felton et al., 1989). As the third step, the dimers are re-oxidized and crosslinked to another polypeptide chain (Arts et al., 2001; Buchner et al., 2006). A schematic representation of the mechanism of phenolic–protein interactions is given in *figure 5*.

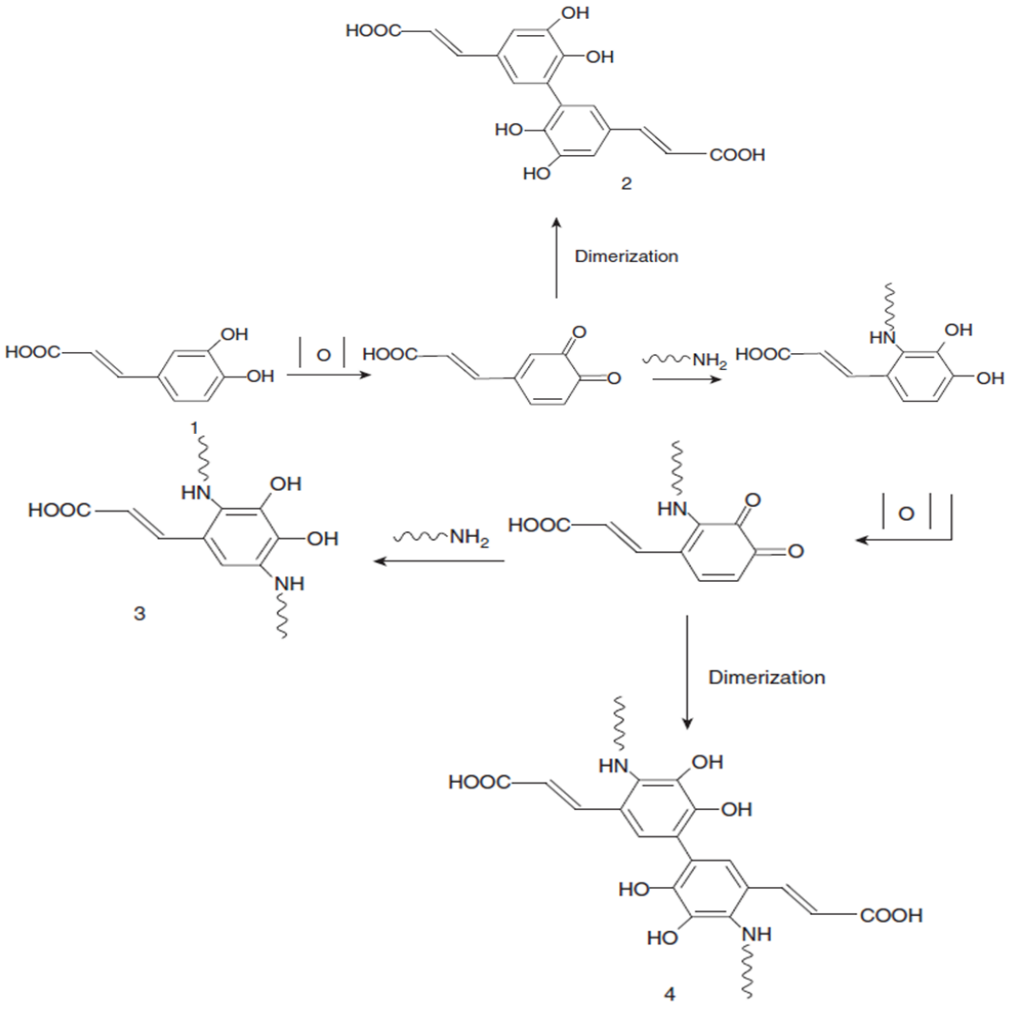


Figure 5: Proposed reactions of a phenolic acid compound with amino side chains of polypeptides (Strauss and Gibson, 2004).

II.3.1. Factors affecting the interactions between proteins and phenolic compounds

While there exist a variety of factors that influence protein-phenolic interactions including environmental factors such as temperature, pH, salt concentration and presence of certain reagents (Kroll et al., 2003; Ozdal et al., 2013), type of protein and structure of phenolic compound have been reported as the two main factors (Czubinski and Dwiecki, 2017).

- **Type of Protein**

The hydrophobicity, isoelectric point and amino acid sequence of proteins influence strongly their binding affinity to phenolic compounds (Prigent et al., 2003). For instance, bovine serum albumin (BSA), which is more hydrophobic than a-lactalbumin and lysozyme, exhibited higher binding affinity to chlorogenic acid (Naczk et al., 1996). Additionally, proteins that have relatively higher molecular weights may exhibit higher affinity for phenolics (Hagerman, 1989). The size of polypeptides and proteins also played a role in their affinities for polymeric proanthocyanidin (Siebert, 1999).

- **Structure of Phenolic Compounds**

Phenolic compounds may differ in their molecular weight, degree of hydroxylation, methylation, glycosylation and hydrogenation, all of which play a role in the formation of protein-phenolic complexes. Polyphenols with higher molecular weights likely have a higher binding affinity to proteins (Dubeau et al., 2010). Compared with some low molecular weight phenolics, protocatechuic acid and caffeic acid showed the strongest binding affinity for BSA whilst p-hydroxybenzoic acid had the lowest (Bartolome et al., 2000). The monoglycoside forms of flavonoids had stronger binding affinities for milk proteins compared to their polyglycoside forms. For quercetin and its glycosides, the aglycone form caused a stronger interaction with BSA (Martini et al., 2008). Methylation was reported to decrease the binding affinity of flavonoids for milk proteins, whereas, hydroxylation of the rings A and B in flavones and flavonols improved the binding affinity. Hydrogenation of flavonoids on their C2 ¼ C3 double bonds led to reduced binding affinities for milk proteins (Xiao et al., 2011).

- **Other Factors**

Temperature is an important environmental factor that influences the binding affinities of phenolic compounds for proteins. The binding affinity of 5-O-caffeoylquinic acid for 11S protein of sunflower seed and BSA decreased with increased temperature (Prigent et al., 2003;

Sastry and Rao, 1990). This was associated with the changes in hydrogen bonding between phenolic compounds and proteins (Sastry and Rao, 1990).

Another influencing environment tal factor is pH. Increased precipitation of protein-polyphenol complexes was observed at pH values close to the isoelectric point of protein, due to a lower solubility of these complexes at this pH. Thus, the optimum pH for precipitation is close to protein isoelectric point, which varies among different proteins (Naczk et al., 1996, 2006). Higher binding affinities of crude tannin canola extract for BSA, fetuin, gelatine and lysozyme, and also, ferulic acid and chlorogenic acid for BSA, were observed all at pHs near the isoelectric points of the proteins (Naczk et al., 1996; Rawel et al., 2005). However, binding of (-)-epicatechin to BSA was not affected by pH (Charlton et al., 2002; Frazier et al., 2006; Papadopoulou et al., 2005).

Protein-phenolic interactions may also be influenced by other environmental factors such as type and concentration of salt. High salt concentrations may lead to dissociation of oligomeric proteins, causing reduced amounts of binding points. Accordingly, increased salt concentration caused a decrease in binding strength of chlorogenic acid to 11S protein of sunflower seed (Sastry and Rao, 1990).

Experimental part

Our experimental approach is summarized in the following diagram

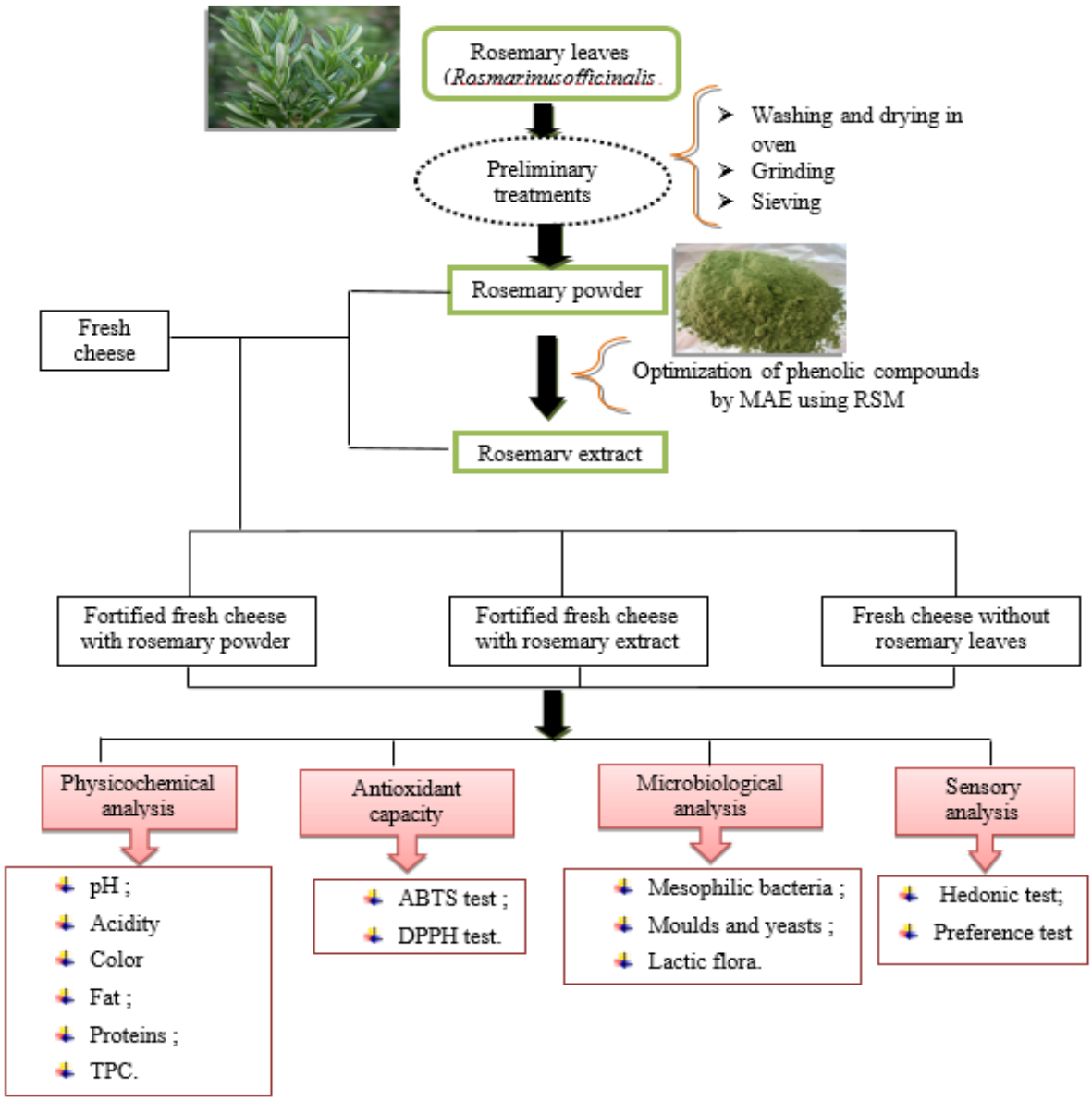


Figure 6. Graphical abstract corresponding to the experimental design

FIRST PART:

Phytochemical analysis of Rosemary

Chapter I:

MATERIALS AND METHODS

MATERIALS AND METHODS

I. Plant material

Leaves of *Rosmarinus officinalis* L.were collected in the area of Aokas (Bejaia, Algeria) in January 2016, washed with tap water and distilled water, dried in a ventilled oven at 40°C for one week, and then reduced into a thin powder using an electrical grinder (IKA model A_{11} Basic). The powder was passed through standard sieves (ø = 125, 250 and 500 µm) and stored in airtight bags under darkness until use.

Before optimization experiments, their initial moisture content was assessed by constant weight at 105°C, and ash content was evaluated by burning (calcination) at 550°C/3hours in a muffle furnace.

II. Optimization of microwave-assisted extraction (MAE)

The extraction of rosemary phenolic compounds was carried out in a domestic microwave oven (MAXMOS23S, Maxipower, China) equipped with a digital control system for irradiation time and microwave power (100-1000W). The apparatus was modified in order to condensate the vapor generated into the sample during the extraction (*Figure 7*).

Figure 7: Microwave oven used for microwave-assisted extraction

A preliminary study (single-factor test) was first analyzed, the different factors evaluated were: particle size (ø = 125, 250 and 500 µm), type of solvent (Water, 80 % ethanol, 80 % methanol, 80 % acetone), solvent concentration (20-95 %), microwave power (100-900 W), solvent to sample ratio (20-140 mL/g DW) and extraction time (30-240 s). Then, the response surface methodology (RSM) with Box- Behnken Design (BBD) was adopted to optimize the extraction of phenolics from *Rosmarinus officinalis* L. leaves powder, of 250 µm diameter using ethanol as solvent. One gram of powder was placed in a round bottom flask of 250mL, and then the solvent was poured into the flasks. At the end of extraction, mixtures were filtered through N°1 Whatman filter paper and extracts were kept in the dark at 4°C until TPC analysis. The effects of four factors on the TPC extraction were studied at three levels -1, 0, +1 (*Table IV*) with three replicates at the center, giving rise to 27 experimental points (*Table V*).

Table IV: Experimental levels of the factors used in BBD for the MAE

Factors	**Levels**		
	-1	0	+1
Ethanol concentration (%, v/v)	40	60	80
Power (W)	300	500	700
Solvent to solid ratio (mL/g)	100	120	140
Extraction time (s)	120	180	240

BBD: Box-Behnken design; MAE: microwave-assisted extraction.

The data from BBD were analyzed to fit the quadratic model given in the following equation (Equation 1):

$$Y = B_0 + \sum_{i=1}^{K} B_i X_i + \sum_{i=1}^{K} B_{ii} X^2 + \sum_{i\rangle j}^{K} B_{ij} X_i X_j + E \qquad (1)$$

Where Y represents the predicted response (TPC); X_i and X_j represent the independent variables, B_0,B_i, B_{ii} and B_{ij} are the regression coefficients for the intercept linear, quadratic and interaction

terms of variables *i* and *j*, respectively. The coefficients B_i, B_{ii} and B_{ij} were determined according to the analysis of variance. An additional extraction trial was achieved under the optimum conditions predicted with the RSM to verify the adequacy of the model; then the experimental data were compared to the predicted ones by the regression model.

Table V: Box-Behnken design for total phenolic compounds of rosemary leaves obtained by microwave-assisted extraction

Run	X_1 (E-OH concentration; %. v/v)	X_2 (Power; W)	X_3 (S/S ratio; mL/g)	X_4 (Extraction time; s)	TPC yield (mg GAE/g DW)	
					Predicted	Experimental
1	40	300	120	180	126.58	126.07
2	40	700	120	180	130.31	129.27
3	80	300	120	180	162.94	163.80
4	80	700	120	180	167.61	167.93
5	60	500	100	120	154.82	152.78
6	60	500	100	240	139.57	140.44
7	60	500	140	120	191.48	190.42
8	60	500	140	240	160.64	162.50
9	40	500	120	120	170.67	172.80
10	40	500	120	240	110.37	109.40
11	80	500	120	120	170.25	171.50
12	80	500	120	240	184.45	182.60
13	60	300	100	180	151.35	148.44
14	60	300	140	180	143.55	142.40
15	60	700	100	180	118.89	120.33
16	60	700	140	180	184.41	187.60
17	40	500	100	180	125.22	126.78
18	40	500	140	180	147.42	146.25
19	80	500	100	180	155.39	156.46
20	80	500	140	180	190.91	189.24
21	60	300	120	120	153.77	155.53
22	60	300	120	240	149.53	151.47
23	60	700	120	120	176.78	174.73
24	60	700	120	240	134.93	133.07
25	60	500	120	180	149.16	148.53
26	60	500	120	180	149.16	149.07
27	60	500	120	180	149.16	149.87

GAE. gallic acid equivalents; dry weight (dw); S/S. solvent to solid; TPC. Total phenolic compounds.

II.1. Spectrophotometric determination of phenolic compounds of rosemary extract

The Folin-Ciocalteu assay was used to determine the ***TPC*** of the obtained extracts according to the method described by Georgé, Brat, Alter, & Amiot (2005). In tubes, 2.5 mL of Folin-Ciocalteu reagent (1/10) were added to 0.5 mL of leaves extracts. After 2 min, 2 mL of 7.5 % (w/v) sodium carbonate (Na_2CO_3) solution was added to each tube, and the tubes were incubated for 15 min/50 °C. After cooling, the absorbance was measured at 750 nm. The TPC was expressed as gallic acid equivalent on dry weight basis (mg GAE/g DW). Gallic acid was used to prepare a calibration curve with different concentrations (20 to 100 µg/mL) using the equation 2:

$$y = 0.12x \quad (R^2 = 0.998) \qquad (2)$$

The optimized extract was also analyzed for the content of flavonoids and tannins:

The amount of ***total flavonoids*** in the extracts was estimated using the methodology of Djeridane et al. (2006). Concentration of flavonoids was expressed as mg of quercétine equivalent per g of dry weight (mg QE/g_{DW}) and obtained from a standard curve prepared with quercétine (5 to 50µg/mL) using 3:

$$y = 28.66x + 0.019 \qquad (R^2 = 0.982) \qquad (3)$$

The content of ***total tannins*** was determined according to the protocol developed by Hagerman and Butler (1978). Content of tannins was expressed as mg tannic acid equivalent per g of dry weight (mg TAE/g_{DW}) using a standard curve prepared with tannic acid at 100 to 1200 µg/mL using the equation 4:

$$y = 1.507x + 0.053 \qquad (R^2 = 0.998) \qquad (4)$$

III. UHPLC-DAD-ESI-MS^n analysis

The rosemary leaves extract obtained above, under the optimum conditions by RSM, was characterized by ultra-high performance liquid chromatography coupled to electrospray mass spectrometry (UHPLC).

To determine individual phenolic compounds, extract samples were filtred through 0.22 nylon filter and analysed by UHPLC-DAD-ESI-MS^n (Marcelo et al, 2015). This analysis was performed on an Ultimate 3000 (Dionex Co., USA) apparatus, composed of a quaternary pump, an autosampler, an ultimate 3000 Diode Array Detector (Dionex Co., USA) and an automatic thermostatic column compartment. The column used was a 100 mm length, 2.1 mm i.d., 1.9 µm particle diameters, end-capped Hypersil Gold C18 column (Thermo Scientific, USA) and its temperature was maintained at 30 °C. Gradient elution was carried out with Acetonitrile (eluent A) and formic acid (eluent B, 1%), both degassed and filtered before use. The gradient solvent used was as follows: 20 min, 90–70% of solvent B; 10 min, 70–40% of solvent B and 5 min, 40–0% of solvent B. The mass spectrometer used was a Thermo LTQ XL (Thermo Scientific, USA) ion trap MS equipped with an ESI source. Control and data acquisition were carried out with the Thermo XcaliburQual Browser data system (Thermo Scientific, USA). Nitrogen above 99% purity was used and the gas pressure was 520 kPa (75 psi). MS analysis was performance with negative voltage of 5 kV and ESI capillary temperature of 200°C with 10-40 arbitrary units of energy collisions in fragmentations.

The identification of individual phenolic compounds in the UHPLC analysis was achieved by comparison of their retention times, UV-Vis spectra and MS^n spectra data with those of the closest available reference standards and data reported in the literature.

The quantification of the main individual phenolic compounds in rosemary extract was performed by the areas of their peaks, through the external standard calibration curves, using the most close reference compounds available.

IV. Biological analyses of rosemary leaves extract

IV.1. Antioxidant capacity

IV.1.1. DPPH radical scavenging capacity

The free radical scavenging capacity using 2,2-diphenyl 1-picrylhydrazyl reagent (DPPH) is based on the reduction of the stable free radical DPPH in the presence of a hydrogen-donating

antioxidant, and the formation of the non-radical form DPPH-H as result of the reaction. This reduction can be monitored at 517 nm by measuring the bleaching of DPPH (violet) to DPPH-H (yellow).

The DPPH test was determined spectrophotometrically in a 96 well plate reader (ELX800 microplate reader) according to the protocole of Marcelo et al, 2015 with some modifications: 250 µL of DPPH ($8.66*10^{-5}$M in ethanol) were added for each well containing 50 µL of extract (2.66-166.67 µg/mL); the microplate was kept in the dark for 30min and the absorbance was measured at 517nm. Ascorbic acid (2.66-166.67 µg/mL) was used as standard.

The radical scavenging activity was calculated as the percentage of DPPH• discoloration according to this formula:

$$\%\ DPPH\ scavenging = \left(1 - \frac{Abs_{c(0)}}{Abs_{e(t)}}\right) X\ 100$$

Where: $Abs_{c(0)}$= Absorbance of the control at t=0, and $Abs_{e(t)}$= Absorbance of the extract at t=30 min

The results were expressed as the inhibitory concentration of the extract/standard needed to decrease DPPH• absorbance by 50% (IC50).

Based on graphic values of percentage of DPPH• inhibition vs. extract concentration, the IC_{50} (concentration of the extract able to inhibit the 50% of the DPPH•) of the rosemary extract was estimated. Ascorbic acid was used as the reference.

IV.1.2. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out as described by Marcelo et al, 2015 with some modifications: 200 µL of rosemary extract (10-125µg/mL) were mixed in tubes with 200 µL of sodium phosphate buffer (0.2M, pH 6.6) and 200 µL of $K_3Fe(CN)_6$(1% w/v). The mixture was incubated in a water bath at 50 C° for 20 min. After incubation, trichloroacetic acid solution (1 mL; 10% w/v) was added and homogenized in the vortex. 75 µL were transferred from each tube to a 96-multiwells plate and 75 µL of water and 15 µL of iron chloride III (0.1% w/v) were

added to each well. Absorbance was measured at 700 nm against a blank. A higher absorbance indicates a higher ferric reducing power of rosemary extract or BHA (10-50 µg/mL) used as positive control.

A linear regression analysis was carried out by plotting the mean absorbance against the concentrations, and the IC_{50} value was determined considering the extract concentration that provides 0.5 of absorbance.

IV.1.3. Nitric oxide (NO) Scavenging assay

The assay was optimized at the QOPNA (Organic Chemistry, Natural Products and Food Stuffs) laboratory of Aveiro University, Portugal. In 96-well microplate, 100 µL of extract at different concentrations (10.42-666.67 µg/mL), solubilized in DMSO, were mixed with 100 µL of SNP solution (Sodium Nitroprusside0.2 mg/mL in potassium buffer, 100mM, pH 7.4); this mixture was incubated under a fluorescent lamp for 15min. After incubation, 100 µL of Griess reagent prepared with equal volume of Griess A (10g/L Sulfanilamide in phosphoric acid 5%) and Griess B (1g/L N-(1-naphthyl) ethylenediamine dihydrochloride in water) were added and the 96-multiwells plate was placed in the dark for 10 min then the absorbance was measured at 562nm against a blank containing all reagents without extract. Extract-colour control was also prepared in the same way using phosphoric acid 5% instead of the DMSO. The standard reference curve was prepared using ascorbic acid (10.42-666.67 µg/mL).

The scavenging capacity is represented as the percentage of NO radical inhibition and was calculated the same way as the percentage of DPPH radical inhibition.

The IC_{50} (concentration of the extract able to inhibit the 50% of the NO) of the rosemary extract and ascorbic acid, taken as positive control, was estimated.

IV.1.4. Scavenging effect on superoxide radical (nonenzimatic assay)

The superoxide anion radical scavenging activity, determined by the phenazonium methosulfate (PMS)-nicotinamide adenine dinucleotide (NADH) generating system is based on the reduction

of nitro blue tetrazolium (NBT) to a purple formazan, in the presence of reduced NADH and PMS under aerobic conditions. The plant extract, when added, will act as a scavenger of the superoxide radicals, competing with NBT preventing the color formation. The superoxide anion radical scavenging activity was optimized at the QOPNA (Organic Chemistry, Natural Products and Food Stuffs) laboratory of Aveiro University, Portugal. Briefly, in 96-well microplate, 75µL NBT (200µM in Potassium Phosphate buffer, 20mM, pH 7.4) were mixed with 100µL NADH (300µM in deionized water), 75µL of extract solution (3.91-250 µg/ml) or gallic acid (0.98-62.5 µg/ml) in DMSO and 50µL PNS (15µM in deionized water). The changes of absorbance at 560nm were recorded over 5min. The IC_{50} (concentration of the extract able to inhibit the 50% of the SO) of the rosemary extract and gallic acid, taken as positive control, was estimated.

IV.2. Inhibition of enzymes

IV.2.1. Inhibition of alpha glucosidase enzyme

α-glucosidase is digestive enzyme located in the brush border of the small intestine that acts upon α (1→4) bonds breaking starch and disaccharides into glucose. In this assay we will use 4-nitrophenyl α-D-glucopyranoside (PNPG) as substrate that develops a yellow color upon hydrolysis.

The reaction kinetics can be determined by measuring absorbances at 405nm. As a reference inhibitor will be using acarbose, an anti-diabetic drug used to treat type 2 diabetes.

The inhibitory activity was determined by incubating, in 96-well plate,50 µL of PNPG solution (17mM) with 50 µL of inhibitor or various concentrations of rosemary extract [prepared in Potassium phosphate buffer, 50mM, pH=6,8 : DMSO (3:2)]. The reaction was started by adding 100 µL of α-glucosidase enzyme (1U/ml) to each well. Then, the absorbance was measured for 20 min at 405 nm. The enzyme activity is determined by calculating the slope of the absorbance at 405 nm versus time in minutes.

The IC_{50} (concentration of the extract/standard able to inhibit the 50% of enzyme) of the rosemary extract and acarbose, taken as enzyme inhibitor, was estimated.

IV.2.2. Assay of Lipase inhibitory activity

The effect of rosemary extract on lipase activity was carried out by mixing 140µL of solution A (prepared in Tris buffer pH=8.4 50mM, 1,8mM sodium deoxycholate and 476 µg/mL colipase) with 30µL solution B (prepared in acetate buffer pH=4,0 18mM, 72mM sodium deoxycholate, 0,2mM calcium chloride, 0,2mM DGGR: 1,2-Di-O-lauryl-*rac*-glycero-3-(glutaric acid 6-methylresorufin ester)). After that, 20µL of rosemary extract or inhibitor solution and 10µL lipase solution were added. After 35 min, the absorbance was measured at 580 nm. The concentration require to give 50% inhibition (IC_{50}) was determined for rosemary extract and orlistat, use as a positive control.

V. Statistical analysis

Data were expressed as mean ± S.D (standard deviation). ANOVA combined with Tukey's test, using XlSTAT 2014, were used to compare significant differences in the biological activities and the content of TPC depending on the solvent and microwave parameters. Differences were considered significant at $p < 0.05$.

Data obtained from the BBD trials for the MAE were statistically analyzed using ANOVA for the response variable in order to test the model significance and suitability. The JMP Pro10 software was used to construct the BBD and to analyze all the results of MAE optimization.

Chapter II:

RESULTS AND

DISCUSSION

RESULTS AND DISCUSSION

I. Moisture and ash content of rosary leaves

The results the determination of the moisture and ash content of fresh rosemary leaves (*Rosemarinus officinalis* L.) are shown in the Table VI. Results of the moisture content are close to those reported by Regiane Ribeiro-Santos et al. (2015) and Bibi Sadia et al. (2016) whom reported 67.77% and 60.39%, respectively. On the other hand, the results obtained of the ash content seem lower than those reported by Kaloustian et al. (2001) and Abayneh Kassahun et al. (2019) whom recovered 6.33% and 7.6%, respectively.

Table VI: Moisture and ash of rosemary leaves

	Moisture content (%)	**Ash content (%)**
Rosemary leaves	63.743 ± 0.091	3.172 ± 0.419

II. Optimization of microwave-assisted extraction

The extraction of phenolic compounds is a crucial step for their valorization. It depends on the method and the appropriate solvent which preserves their biological properties. Because of the effect of various parameters on the extraction yield by MAE, the optimization of the experimental conditions is a critical step. The range of extraction parameters chosen in this study (Table IV) was based on the preliminary experiments; the response surface model was fitted based on the TPC yield (Table VII) which varied from 109.40 to 190.42 mg GAE/g DW. Statistical analysis has been carried out to determine the significance of the regression model which was checked by *F-test* and *p-value*; the analysis of variance (ANOVA) and fitting results are shown in Table VII.

Based on the statistical and fitting analysis, the predicted model can be described in terms of coded significant factors as in the equation bellow:

$$TPC = 149.15 + 18.41X_1 + 2.10X_2 + 14.43X_3 - 11.52X_4 + 3.32X_1X_3 + 18.32X_2X_3 + 18.62X_1X_4 - 9.40X_2X_4 - 3.89X_3X_4 - 3.74X_2^2 + 4.13X_3^2 + 8.33X_4^2 \quad (5)$$

It can be seen that there were linear, interactive and quadratic effects between all the extraction factors except the quadratic effect of the solvent (X_1^2), and its interaction with microwave power (X_1X_2). The interaction solvent-time and power-solvent to solid ratio were highly significant as well as the quadratic term of time.

Table VII: Analysis of variance (ANOVA) for the experimental results obtained by microwave-assisted extraction

Parameter[a]	Estimated coefficients	Standard error	DF[b]	Sum of squares	F-value	P-Value Prob> F
Model	149.15667	1.367854	14	12087.233	153.8147	<.0001
Intercept						
B_0	149.15667	1.367854	1			
Linear						
X_1-Solvent	18.413333	0.683927	1	4068.6101	724.845	<.0001
X_2-Power	2.1016667	0.683927	1	53.004	9.443	0.0097
X_3-Ratio	14.431667	0.683927	1	2499.276	445.2596	<.0001
X_4-Time	-11.52333	0.683927	1	1593.4465	283.8811	<.0001
Interaction						
X_1X_2	0.2325	1.184597	1	0.2162	0.0385	0.8477
X_1X_3	3.3275	1.184597	1	44.289	7.8903	0.0158
X_1X_4	18.625	1.184597	1	1387.5625	247.2018	<.0001
X_2X_3	18.3275	1.184597	1	1343.589	239.3677	<.0001
X_2X_4	-9.4	1.184597	1	353.44	62.9673	<.0001
X_3X_4	-3.895	1.184597		60.6841	10.8112	0.0065
Quadratic						
X_1^2	1.4420833	1.025891	1	11.0912	1.976	0.1852
X_2^2	-3.740417	1.025891	1	74.6172	13.2935	0.0034
X_3^2	4.1345833	1.025891	1	91.1722	16.2428	0.0017
X_4^2	8.3345833	1.025891	1	370.4815	66.0033	<.0001
Lack of fit			10	66.44785	14.6189	0.0657
Residual			12	67.357		
Pure error			2	0.909067		
R^2	0.994458					
R^2ajusted	0.987993					
RMSE	2.369193					
Total Corr.			26	12154.59		
C.V (%)	1.99					

a: Coefficients refer to the general model; b: Degree of freedom; C.V: Coefficient of variation

According to the results in Table 7 (*F*-statistic, *P*-value, lack of fit, coefficient of determination: R^2 and the coefficient of variation: C.V); it can be suggested that the obtained results adequately fitted the selected equation and the model was highly significant: *P-values* of the developed

model were below 0.0001 which means that the fitness of the model was highly significant ($p < 0.0001$), the adjusted determination coefficient (R^2_{Adj}) was quite close to R^2; the value of lack of fit test (0.0657) is higher than 0.05 and the low value of the coefficient of variation (CV = 1.99) suggested that the model was reliable and reproducible,

To better understand the resulting predictive model, three-dimensional response surface plots of rosemary TPC are illustrated in Figure 8. This plotting is very useful to visualize the effect of two factors on the response at the same time (Karabegović et al., 2014). The graphs were designed by plotting the response using the Z-axis against two independent variables; the other two independent variables were kept at their zero level. The linear effects of solvent, power and ratio of solvent to raw material were positively significant, and then by increasing them the extraction yield is increased (Figure 8, B, D). It can be seen that the extraction time has also an effect with its interaction with the solvent and the solvent to solid ratio (Figure 8, C, F): with an increase in ethanol/water solvent or solid/ethanol ratio and decreased extraction time, the recovery of TPC was increased. Several studies reported the influence of solvent and MAE conditions on the extraction of phenolic compounds from vegetable tissues (Bai, Yue, Yuan, & Zhang, 2010; Karabegović et al., 2013; Pan, Niu, & Liu, 2003; Švarc-Gajić et al., 2013; Zhang et al., 2013). then it can be used for optimization of rosemary TPC extraction (Karabegović et al., 2014; Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2013; Maran et al., 2014; Yang et al., 2009; Yu et al., 2013; Zhang et al., 2013).

In order to validate the accuracy of the model, rosemary powder was extracted under the recommended optimum conditions, determined using the RMS optimization approach, which were as follows: ethanol concentration 78.162%, microwave power 351.825W, ratio of solvent to raw material 101.623:1, and extraction time 122.648s.

The mean experimental TPC yield (150.157±1.18 mg GAE/g DW) was close to the predicted results (149.156 ±2.98 mg GAE/g DW).

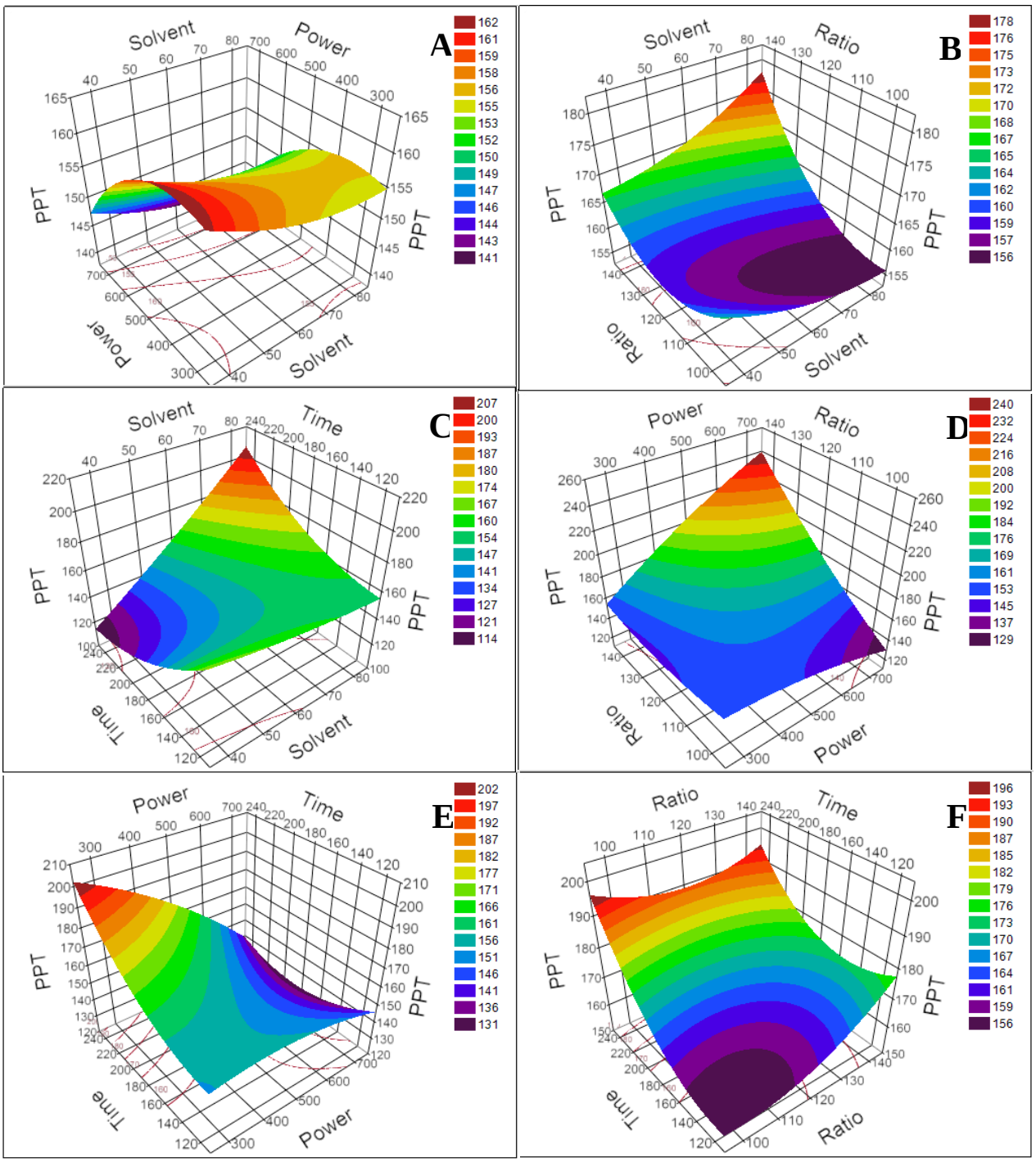


Figure 8: Microwave-assisted extraction parameters effect on total phenolic yield from *Rosmarinus officinalis* L. leaves with respect to ethanol percentage and microwave power **(A)**; ethanol percentage and solvent-to-solid ratio **(B)**; ethanol percentage and irradiation time **(C)**; solvent-to-solid ratio and microwave power **(D)**; microwave power and irradiation time **(E)**; solvent-to-solid ratio and irradiation time **(F)**. ***PPT:*** Total polyphenols of rosemary extract (mg_{GAE}/g_{DW}); ***Solvent:*** Ethanol (%); ***Power:*** Microwave power (W); ***Ratio:*** Solvent to solid ratio (mL/g); **Time:** Irradiation time (s).

III. Rosemary phenolics and characterization of phenolic compounds

The total phenolic content of the rosemary extract amounted to 150.157 ± 1.18 mg GAEq/g_{DW}, its flavonoids value was 54.271 ± 2.202 mg QE/g_{DW} and its tannins content was 32.487 ± 3.607 mg TAE/g_{DW}. *Rosemarinus officinqlis* L. leaves were found to be a rich source of polyphenolic compounds, thus qualifying this spice as a potential source of phenolics and flavonoids.

The rosemary leaves extract was analyzed by ultra-high performance liquid chromatography coupled to electrospray mass spectrometry (UHPLC–DAD–MS^n). Afterwards, the compounds characterized were quantified in the extract using commercialized standards available.

The analysis of rosemary extract revealed 12 major compounds, mainly flavonoids and phenolic acids and diterpens (Table VIII and Figure 9). The detected compounds were characterized by comparison of retentiontimes (RT), UVmax, [M–H]-ion and the MS/MS fragment ions. The remaining identifications were performed by interpretation of the MS and MS/MS spectra of the detected compounds combined by the data from the literature and data bases. The fragmentation patterns of these compounds have been previously identified in other works (Borras Linares I. et al., 2011; Ivanivic (2009); Santana-Méridas O. et al., 2014; Vallverdu-Queralt A. et al., 2014).

Table VIII: Compounds characterized in rosemary-leaf extract.

Peak	Retention time (min)	UVmax (nm)	[M–H]⁻	MS^2 ions	Compounds
1	12.61	337	477	315, 300	Nepitrin
2	13.85	328	359	161, 197, 179,	Rosmarinic acid
3	14.14	322	461	223	Luteolin 3-glucoronic
4	14.56	316	623	285	Kaempferol-hesoside-glucoronique
5	15.20	322	503	285, 323, 299	Luteolin 3'-O-(O-acetyl)-β-D-
6	15.54	322	521	285, 399	glucuronide Isomer I
7	20.03	277	345	313	Rosmarinic acid-O-hexoside
8	20.36	244	345	301	Rosmanol
9	21.65	284	285	301, 283	Epi-rosmanol
10	21.80	267	329	285, 270, 201	Carnosol
11	22.24	284	331	285	Carnosol isomer
12	22.52	277	345	287	Carnosic acid
				301, 286	Methoxy-carnosic acid

The main compounds in the studied herb extract were terpenoïds: carnosol (C), carnosic acid (CA), 1, 2, methoxy-carnosic acid (MCA) and rosmanol (R), phenolic acid as rosmarinic acid (RA), while other phenolics, such as flavonoids (kaempferol and luteolin), appeared as minor compounds.

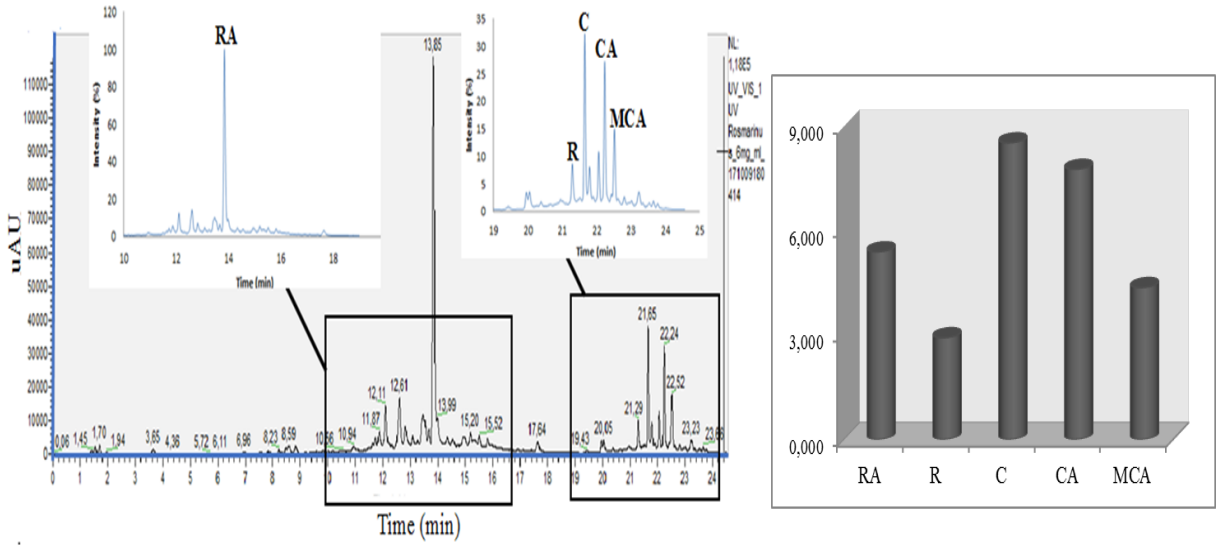


Figure 9: Chromatographic profile of the rosemary leaves extract with quantitative characterization of the prominent compounds. *RA: rosmarinic acid, R: rosmanol, C: carnosol, CA: carnosic acid, MCA: 1,2 methoxy-carnosic acid, the quantification was expressed in mg of compound per g of rosemary extract.*

The compound concentrations were determined using the corrected area of each individual compound and by interpolation in the corresponding calibration curve. The quantitative results (Figure 9) showed that the most prominent compound in the rosemary extract found to be carnosol and carnosic acid, which presented 8.535 and 7.774 mg/g of extract, respectively, followed by rosmarinic acid which presented 5.400 mg/g of extract. According to previously published results of Borras Linares I. et al. (2014) and Almela et al. (2006), the most abundant compounds in the rosemary-leaf extracts were phenolic diterpenes, specifically carnosic acid and carnosol, and phenolic acid (rosmarinic acid).

IV. Biological activities of rosemary extract

Rosemarinus officinalis and its main polyphenols have been the topic of many studies related to several effects. In this part of study, the antioxidant and antidiabetic activities of the rosemary ethanolic extract were carried out by different methods.

IV.1. Antioxidant activity

Oxidative stress has been implicated in numerous pathologic conditions, such as inflammation, diabetes, cardiovascular diseases, cancer, and ageing, while natural fractions are potential sources of bioactive compounds able to counteract such events. To provide evidence of the antioxidant potential of the rosemary ethanolic extract, four in vitro essays (DPPH, FRAP, NO, SO) were conducted. The results are shown in the Table VIIII.

Table VIIII: Antioxidant activities of rosemary extract and standards expressed by IC_{50} (µg/mL)

Antioxidant activities							
DPPH		***FRAP***		***NO***		***SO***	
Ascorbic acid	Rosemary extract	BHA	Rosemary extract	Ascorbic acid	Rosemary extract	Gallic acid	Rosemary extract
11.749±0.33	18.636±0.13	21.376±0.59	85.879±0.45	27.947±0.28	39.018±0.36	5.013±0.04	75.608±0.22

The scavenging DPPH method uses a stable radical, DPPH, which give deep purple color. By addition of DPPH, the color of the solution fades and the reduction is monitored by the decrease in the absorbance at 515 nm. When a solution of DPPH is mixed with a substance that can donate an hydrogen atom, the reduced form of the radical is generated accompanied by loss of color. This delocalization is also responsible for the deep violet color, characterized by an absorption band at about 515 nm.

Scherer and Godoy (2009) described the Antioxidant Activity Index (AAI) as: AAI = final concentration of DPPH (µg/mL) / IC_{50} (µg/mL). This index classifies antioxidants as: weak AAI < 0.5; moderate 0.5 < AAI < 1.0; strong 1.0 < AAI < 2.0; and very strong AAI > 2.0. The

final concentration of DPPH used was 34 µg/mL then, rosemary extract was considered strong antioxidant (AAI = 1.825). Furthermore, the rosemary extract exhibited activity comparable to that of ascorbic acid (AAI = 2.893).

The FRAP assay measures the reduction of a ferric salt to a blue colored ferrous complex by antioxidants under acidic condition. The FRAP assay is a simple, economic and reducible method which can be applied to plant extracts. As shown in Table VIIII, the rosemary extract has the capacity to reduce ferric iron (Fe^{+3}) to ferrous iron (Fe^{+2}), but this ability was four times less than BHA.

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured using the Griess reaction reagent (1% sulphanilamide, 0.1% naphthyethylene diamine dihydrochloride in 2% H3PO3). Nitric oxide ($NO^{\cdot}$) has been recognized as a versatile player in several biological mechanisms, including endothelial cell function and inflammation, turning it into a biomarker in the screening of new anti-hypertensive and anti-inflammatory drugs. Therefore, the potential capability of the aqueous extracts from apple pomace to regulate NO_-driven processes was inferred through its ability to scavenge chemically generated $NO^{\cdot}$.

As represented in Table VIIII, the rosemary extract was able to inhibit the nitrite oxide generated and exhibited activity comparable to that of ascorbic acid.

Superoxide anion generates powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress. In the PMS/NADH-NBT system, the superoxide anion derived from dissolved oxygen from PMS/NADH coupling reaction reduces NBT. The data illustrated in Table VIIII showed that rosemary extract is stronger superoxide anion quencher but this inhibition was, however, lower than that produced by gallic acid.

Many scientific articles have correlated the antioxidant activities of rosemary extracts with the presence of specific polyphenol components. For example, carnosic acid was considered by Klanˇcnik et al. (2010); Bubonja-Sonje et al. (2011); Piskernik et al. (2011) and Vegara et al.

(2011) as the major contributor to rosemary's antioxidant activity. Others authors, such as Cui et al. (2012) and Kontogianni et al. (2013), considered that carnosol, by itself, has an important specific bearing on the antioxidant capacity of rosemary extracts. Rosmarinic acid has also been defined as good radical scavenger by several authors, including Hosseinimehr et al. (2009); Yehb et al. (2009); Ahmad et al. **(**2012); Souza et al. (2013); Yang et al. (2012) and Yang et al. (2013). As regards our results (Figure 9), several components, including carnosol (C), carnosic acid (CA) and rosmarinic acid (RA), showed higher concentrations in the rosemary extract, and it was the greater presence of these components that was responsible for the increased antioxidant capacity of the corresponding extract.

Table X: Correlation between the antioxidant properties and phytochemicals of rosemary leaves extract

Variables	TPC	TF	RA	C	CA	FRAP	DPPH	NO	SO
TPC	**1**								
TF	0.154	**1**							
RA	0.225	0.997	**1**						
C	0.293	-0.900	-0.866	**1**					
CA	0.406	0.965	0.982	-0.755	**1**				
FRAP	0.176	1.000	0.999	-0.890	0.971	**1**			
DPPH	0.342	0.981	0.993	-0.799	0.998	0.985	**1**		
NO	0.126	1.000	0.995	-0.912	0.958	0.999	0.975	**1**	
SO	1.000	0.154	0.225	0.293	0.406	0.176	0.342	0.126	**1**

***TPC**: total phenolic content, **TF**: total flavonoids, **RA**: rosmarinic acid, **C**: carnosol, **CA**: carnosic acid.*

Several studies have reported a high correlation between the antioxidant effect of plant extracts with the content of phenolic compounds. The correlations between antioxidant activity and

contents of phytochemicals of rosemary extract are analyzed (Table X). For scavenging effects on radicals and FRAP, high correlations (r = 0.975–0.999) were observed among FRAP, DPPH and nitric oxide (NO), indicating that these three methods have satisfactory correlations for the examination of antioxidants.

The scavenging effects on free radicals (DPPH, NO) and FRAP of rosemary extract were also correlated well with its content of total flavonoids (r = 0.981–1) and individual phenolics (r = -0.799-0.999), while the total phenolic content showed a very lower degree of correlation. However, significantly correlation for scavenging effect on superoxide (SO) was only observed with total phenolic content.

IV.2. Antidiabetic activity

In this part of study, the effects of ethanolic extract of *Rosmarinus officinalis* on α-Glucosidase and Anti-lipase activities were investigated.

Table XI: Inhibitory activities of rosemary extract towards *α*-glucosidase and lipase (expressed as IC_{50})

Antidiabetic activities			
α-Glucosidase		***Anti-lipase***	
Acarbose	Rosemary extract	Orlistat	Rosemary extract
591.75 ± 0.591	388.66 ± 0.951	0.002 ± 0.004	273.209 ± 0.017

As shown in Table XI**,** the plant extract exhibited a significant inhibitory activity on enzymes, *α*-glucosidase and lipase: α-Glucosidase activity of the rosemary extract was stronger than that of the commercial drug acarbose and overall, this extract showed a quite good result against lipase.

Table XII: Correlation between the antidiabetic activities and phytochemicals of rosemary extract

Variables	TPC	TF	RA	C	CA	α-glucosidase	Anti-lipase
TPC	**1**						
TF	0.154	**1**					
RA	0.225	0.997	**1**				
C	0.293	-0.900	-0.866	**1**			
CA	0.406	0.965	0.982	-0.755	**1**		
α-glucosidase	0.469	-0.800	-0.755	0.982	-0.616	**1**	
Anti-lipase	0.120	-0.962	-0.940	0.984	-0.859	0,933	**1**

When comparing the total phenolic concentration of the rosemary extract with the α-glucosidase and lipase inhibitory activities no correlation was observed. However, high correlations were observed among these inhibitory activities and total flavonoids (TF), rosmarinic cid (RA), carnosol (C) and carnosic acid (CA) (Table XII**).**

Madina Naimi et al. (2017),reported that rosemary extract and rosemary extract polyphenols exhibit protective properties against hyperlipidemia and hyperglycemia in genetic, chemically-induced and dietary animal models of obesity and type 2 diabetes mellitus. They suggested a potential to use rosemary extract and its polyphenolic constituents towards the management of blood glucose levels and diabetes.

SECOND PART:

Enrichment of cheese by rosemary

Chapter I:

MATERIALS AND METHODS

MATERIALS AND METHODS

I. Impact of rosemary addition on physicochemical parameters, sensory attributes and microbiological quality of rosemary-supplemented cheese

I.1. Preparation of rosemary-supplemented cheeses and sampling

The fresh cheese used in this part of study was manufactured at the Sittelle Kabyle SARL in Aokas (Bejaia, Algeria) in June, 2017. After draining, the curd was aseptically supplemented with rosemary leaves powder (Cheese B: 0.25 %; Cheese C: 0.5 % and Cheese D: 0.75 %) or with their lyophilized ethanolic extract (Cheese E: 0.25 % and Cheese F: 0.5 %), and put into sterile boxes. Before adding the powder and the extract, they were first sterilized at 90°C/5min. The rosemary fortified-cheeses and control cheese (Cheese A) were kept at 5 ±1 °C until analysis of some physicochemical characteristics (pH, acidity, fat, and proteins), sensory appreciation and microbial quality. Sampling was performed after storage for 1 day (T1), 5 days (T2), 10 days (T3), 14 days (T4), and 18 days (T5).

I.2. Physicochemical and compositional analyses

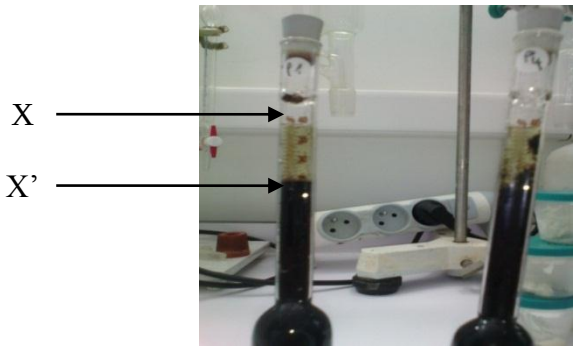
The pH was measured in cheese samples prepared by homogenizing 5 g of each sample with 45 mL of distilled water.

For determination of titratable acidity, 2g of cheese were crushed with 20mL of distilled water in porcelain mortar. This solution was transferred into an erlenmayer flask, 5 drops of phenolphthalein was added and titrated with 0.1N NaOH to the first permanent color change to pink.

$$Acidity\ (°D) = NaOHamount * 0.9 * 10$$

The fat content was determined by the Gerber method (ISO 1211), 3 g cheese sample was weighed into a butyrometer vessel and filled with 10 mL H2SO4 (70%) to be incubated 60min/80°C.

After incubation, 1 mL amyl alcohol was added. Butyrometer vessel was completed to the level of 35% with H2SO4 solution and centrifuged in Gerber centrifuge for 10 min. The oil level was read from butyrometer vessel.



The proteins concentration in cheeses was quantified by the biuret method using bovine serum albumin as a standard (Gornall et al., 1949).

I.3. Microbiological Analysis

After adding rosemary leaves to the cheese, yeasts and molds as well as the total coliforms were counted on D+1 (the second day of storage).

In order to assess the effect of rosemary addition on lactic bacteria, cheese samples were followed up for 18 days of storage.

The direct contact method (mass inoculation) was adopted.

Before opening the cheese box (10g), and in order to eliminate any source of contamination, its outer surface was carefully cleaned with ethanol. The container was aseptically opened and homogenized with 90mL of 0.1 % sterile peptone water. Serial 8 fold dilutions in sterile 0.1 % peptone water were prepared for bacterial analysis. Two measurements were carried out and average values are represented. The sterility of the agars and peptone water was checked.

Total Aerobic Count: Plate Count Agar was used for total aerobic count. Plates were incubated at 30°C for 72 h.

Yeast and Mould Enumeration: Oxytetracycline Glucose Agar was used for yeast and mould enumeration. Plates were incubated at 25°C for 5 days.

Coliform Bacteria Enumeration: Violet Red Bile Agar was used for the enumeration of coliforms. Plates were incubated at 37°C for 24 h.

***Lactobacillus* Enumeration:** MRS agar was used for the enumeration of *Lactobacillus*. Plates of dilutions 10^{-4}, 10^{-5} 10^{-6} and 10^{-7}were incubated at 30°C for 24 h.

***Lactococcus* Enumeration:** M 17 Agar was used for the enumeration of *Lactococcus*. Plates of dilutions 10^{-4}, 10^{-5} 10^{-6} and 10^{-7}were incubated at 30°C for 24 h.

I.4. Sensory analyses

Cheese samples (fortified and control) were submitted to sensory analysis (hedonic test). This analysis was conducted at the Laboratory of sensory analysis of Bejaia-University; the cheeses were stored (4 °C), and were previously analyzed on the first day of storage for microbiological sanitary parameters (total coliforms, thermotolerant coliforms), thus promoting the safety of the panelists.

Ten panelists (n = 10; including five females and five males, aging from 27 to 50 years), familiar with sensory evaluation techniques and regular consumers of food products, evaluated the sensory quality of the prepared samples (Djaoud et al., 2020a; Wang, Liu, Jing, Fan, & Cai, 2019). They were asked to score the color, odor, acidity, aroma, smooth texture, aftertaste and the overall acceptability. The sensory analysis was based on a five-point scale for hedonic measurements; for color: 1. White, 2. Beige, 3.Yellow-green, 4. Light-green, 5. Blue-green ; for the other sensory descriptors : 1. Absence, 2. Low, 3. Average, 4. Strong, 5. Very strong, and extremely unpleasant (1 point) or extremely pleasant (9 points) were used for scoring panelists preferences (acceptability). Cheese samples (15 g in weight), at the 2nd day of storing, were randomly presented in transparent and individual plastic cups coded with 3-digit numbers, at room temperature. Water and bread were used as neutralizing agents during samples tastings.

II. Effect on color, phenolic compounds and antioxidant activity of rosemary fortified fresh cheese

II.1. Preparation of rosemary-supplemented cheeses and obtaining of the respective extracts

The fresh cheese used in this part of study was produced at the Agrarian Higher School (pilot scale) of Coimbra, Portugal in October, 2018. After draining, the curd was aseptically supplemented with rosemary leaves powder (cheese C:0.5% and cheese G:1%) or with hydroethanolic extract (cheese E:0.25% and cheese F:0.5%) and kept into sterile boxes. The rosemary fortified-cheeses and control (cheese A) were left at 5°C until analysis, that were performed after storage for 1 day (T1), 5 days (T2), 8 days (T3), 12 days (T4) and 16 days (T5). Cheese extracts (to be used in for analysis of phenolic compounds and antioxidant capacity) were prepared according to the procedure of Fernandes, Ferreira, Bastos, Ferreira, Cruz, Pinto, et al., (2019) with some modifications: five grams of cheese were stirred for 4 h with 50 mL of methanol acidified with formic acid (9:1 v/v). The mixture was kept overnight at -18°C then centrifuged (6000 rpm/30 min/4°C) and filtered through a G4 membrane to remove soluble proteins. The samples were kept in the freezer until analysis.

II.2. Surface color

The cheese samples (control and rosemary-fortified) were characterized regarding color, as well phenolic compounds.

Surface color was determined with a colorimeter (CM 2300d, Konica Minolta, Japan) through coordinates CIELAB a* (+ red, - green), b* (+ yellow, - blue) and L* (darkness-lightness). Total color difference (ΔE*) was determined as previously described by Pathare, Opara, and Al-Said (2013) and Patras, Brunton, Tiwari, and Butler (2011):

$$\Delta E = \sqrt{(a^* - a_0)^2 + (b^* - b_0)^2 + (L^* - L_0)^2}$$

Where a_0, b_0 and L_0 are the coordinates of cheese control.

II.3. Quantitative and qualitative analysis of phenolic compounds

The content of total phenolic compounds (TPC) was determined using the Folin-Ciocalteu reagent as previously described by Marcelo et al. (2015) with some modifications: in tubes, 60 µL of cheese extracts were added to 2.34 mL of distilled water and 150 µL Folin-Ciocalteu reagent. After 8 min of incubation, 450 µL of 30% (w/v) sodium carbonate (Na_2CO_3) solution was added to each tube. These were then incubated for 30 min in the dark and the absorbance was measured at 750 nm. Gallic acid was used as standard and TPC concentration was expressed in mg of Gallic Acid Equivalents (GAE) per g of fresh cheese.

To determine individual phenolic compounds, extract samples of cheese were filtred through 0.22 nylon filter and analysed by UHPLC-DAD-ESI-MS^n (Fernandes, Ferreira, Bastos, Ferreira, Cruz, Pinto, et al., 2019), as described in the previous chapter.

II.4. Antioxidant capacity of cheese samples

The antioxidant capacity of cheeses was screened by the ABTS assay, as described by Zeyneb et al. (2020) with some modifications: Cheese extracts (50 µL) were added to 250 µL of diluted $ABTS^{\bullet+}$ solution (until its absorbance reaches 0.75-0.80). After 15 min of incubation in dark conditions, the absorption at 734 nm was measured using an ELX800 microplate reader. The inhibition (%) of cheese samples was then compared with a standard curve made from the corresponding readings of ascorbic acid (0.02–0.50 mg/mL) and results expressed as mg ascorbic acid equivalents/g fresh weight.

III. Statistical analysis

Results of each analysis were expressed as mean ± standard deviation. All data were subjected to an analysis of variance (ANOVA) and Tukey's multiple comparison tests, considering a 95% significance level.

Principal components analysis (PCA) was performed to explore the differences between cheese samples and the relationship between ABTS and rosemary polyphenols. Excel Stat 2014 Statistical Software was used to run the analyses.

Chapter II:

RESULTS AND

DISCUSSION

In this part, rosemary powder and RSM-optimized extract (obtained above) were added to the fresh cheese at different concentrations to see whether or not this fortification would have an effect on the different physicochemical, lactic flora and sensory parameters.

I. Physicochemical parameters of cheese extracts

I.1.Rosemary-cheese supplementation effect on pH, acidity, proteins and fat contents

Results of rosemary addition on physicochemical parameters of cheese samples are shown on the table below.

Table XIII: Changes in physicochemical parameters of samples throughout storage

	Time (days)	Cheese A	Cheese B	Cheese C	Cheese D	Cheese E	Cheese F
pH	T1	6.18 ± 0.08^{aA}	6.19 ± 0.13^{aA}	6.18 ± 0.15^{aA}	6.19 ± 0.06^{aA}	6.17 ± 0.06^{aA}	$6,18 \pm 0.12^{aA}$
	T2	5.80 ± 0.11^{bA}	6.00 ± 0.08^{aA}	6.02 ± 0.08^{aA}	6.00 ± 0.12^{aA}	6.05 ± 0.08^{aA}	6.01 ± 1.12^{aA}
	T3	5.02 ± 0.07^{cA}	5.76 ± 0.08^{bC}	5.37 ± 0.17^{bB}	5.44 ± 0.15^{bB}	5.43 ± 0.16^{bB}	5.58 ± 1.21^{bC}
	T4	4.72 ± 0.12^{dA}	5.05 ± 0.12^{cB}	5.19 ± 0.07^{cB}	5.17 ± 0.06^{cB}	5.15 ± 0.11^{cB}	5.16 ± 0.08^{cB}
	T5	4.41 ± 0.08^{dA}	4.54 ± 0.10^{dA}	4.65 ± 0.07^{dA}	4.67 ± 0.10^{dA}	4.56 ± 0.07^{dA}	4.66 ± 1.23^{dA}
Acidity (°D)	T1	65 ± 0.16^{aA}	64 ± 0.12^{aA}	63 ± 0.17^{aA}	64 ± 0.19^{aA}	65 ± 0.08^{aA}	63 ± 1.25^{aA}
	T2	70 ± 0.31^{bA}	76 ± 0.25^{bB}	77 ± 0.21^{bB}	75 ± 0.11^{bB}	78 ± 1.23^{bB}	74 ± 0.08^{bB}
	T3	124 ± 0.25^{cA}	123 ± 0.16^{cA}	122 ± 0.06^{cA}	121 ± 0.12^{cA}	124 ± 0.12^{cA}	119 ± 1.21^{cA}
	T4	160 ± 0.38^{dA}	158 ± 0.12^{dA}	159 ± 0.12^{dA}	155 ± 0.21^{dA}	159 ± 0.21^{dA}	156 ± 2.12^{dA}
	T5	190 ± 0.12^{eA}	188 ± 0.08^{eA}	189 ± 0.32^{eA}	185 ± 0.12^{eA}	189 ± 1.25^{eA}	186 ± 1.22^{eA}
Proteins (mg/mL)	T1	5.20 ± 0.12^{aA}	5.40 ± 0.11^{aA}	5.30 ± 0.08^{aA}	5.70 ± 0.15^{aB}	5.70 ± 0.21^{bB}	5.75 ± 0.15^{bB}
	T2	5.00 ± 0.21^{aA}	5.50 ± 0.16^{aB}	5.30 ± 0.12^{aA}	5.60 ± 0.12^{aB}	5.40 ± 0.18^{aA}	5.55 ± 0.18^{bA}
	T3	5.15 ± 0.15^{aA}	5.50 ± 0.16^{aB}	5.50 ± 0.18^{bB}	5.62 ± 0.16^{aB}	5.60 ± 0.21^{bB}	5.70 ± 0.21^{bB}
	T4	5.10 ± 0.22^{aA}	5.40 ± 0.15^{aA}	5.30 ± 0.18^{aA}	5.50 ± 0.15^{aB}	5.50 ± 0.22^{aB}	5.60 ± 0.12^{bB}
	T5	5.18 ± 0.18^{aA}	5.25 ± 0.21^{bA}	5.15 ± 0.22^{aA}	5.30 ± 0.18^{aA}	5.20 ± 0.12^{aA}	5.32 ± 0.22^{aA}
Fat (%)	T1	15^{aA}	14^{aA}	14^{aA}	14^{aA}	15^{aA}	14^{aA}
	T2	15^{aA}	14^{aA}	14^{aA}	16^{aA}	15^{aA}	15^{aA}
	T3	15^{aA}	15^{aA}	13^{aA}	14^{aA}	15^{aA}	15^{aA}
	T4	15^{aA}	14^{aA}	14^{aA}	14^{aA}	15^{aA}	14^{aA}
	T5	15^{aA}	14^{aA}	14^{aA}	16^{aA}	15^{aA}	15^{aA}

Different letters indicate significant differences ($p < 0.05$) according to Tukey´s test. T1. T2. T3. T4 and T5 correspond to 1. 5. 10, 14 and 18 days of storage, respectively.

Overall, no significant differences ($p > 0.05$) were registered among the different cheese samples regarding pH [6.19 – 4.41], the acidity [63 – 190 °D], the protein percentage [5.00 - 5.70 mg/g] and the fat percentage [14 - 16 %].

The pH of cheese samples, in the 1st day (T1), ranged from 6.17 to 6.18, values that are similar to that described by Silva et al. (2010), who reported pH (5.99-7.3) in Coalho cheese made from cow's milk marketed in the Brazilian Northeast. The pH of fresh cheese ranges from 5.1 to 5.5 and the Dornic acidity can be between 65 and 80°D (FAO, 2012).

According to Mahaut et al. (2000), the pH and the acidity can be influenced by several factors, such as the load microbial initial of milk and hygienic handing conditions. In a study carried out on different fresh cheeses from different regions, Jimenez-Maroto et al. (2016) showed that moisture influences the pH value which ranged from 5.3 to 6.5 for high water content. Also in agreement with other authors (Rita Ramos et al., 2013; Santa et al., 2013), the pH of control and fortified cheeses tented to decrease over the storage time with significant differences ($P < 0.05$). However, no significant differences ($P > 0.05$) of pH values among the different cheeses. According to (Rita Ramos et al. (2013), pH values of cheeses are related to the time of ripening because ripened cheeses present lower moisture and higher acidity than unripened cheeses. However since our cheeses were not ripened few significant variations of such parameter were observed throughout storage time.

The buterometric method was used to determine the fat content in fresh cheese samples and the results obtained ranged from 14 to 16g of fat per 100g of fresh cheese. According to Luquet (1990), the fat content in a fresh cheese must be less than or equal to 20g per 100g of fresh cheese after draining.

I.2. Surface color evolution

Color is one of major factors which affect a consumer's choice and selection of cheese, and it is also a powerful tool for quality control and marketing.

The effect of rosemary (powder/extract) fortification on the surface color of the cheeses was evaluated throughout the storage days and compared to the control. Note that in the CIEL*L*a*b* system, a* is a negative or positive value for greenish or reddish colors, respectively, whereas b* assume negative values for the bluish tonalities, and positive values for yellowish ones. L* represents an approximate measurement of luminosity, which is the property according to which each color could be considered as equivalent to a member of the grey scale, between black and white (Granato & Masson, 2010; Pathare, Opara, & Al-Said, 2013). In general, the supplementation with rosemary powder or extract, at levels of 0.5/1% and 0.25/0.5%, respectively, significantly changed the color attributes of the cheeses, while these were not affected during the storage time (Table 14). All the samples presented high values of lightness, meaning that the cheese samples were bright, albeit fortification with rosemary (and particularly with powder), reduced it. In addition, b* coordinate was strongly impacted with the rosemary supplementation, with the yellowness tones being intensified, particularly with adding of the hydroethanolic extracts (Δb* of 3.1-5.7 and 8.2-11.2 for cheeses E-F and C-G, respectively). This fact may in part occur do to the presence of yellow or brownish compounds (eg. flavonoids, carotenoids, degraded chlorophylls), which are expected to be concentrated in the extracts.

Total color difference (ΔE*) is a key parameter for the assessment of magnitude of colordifferences between control and fortified cheeses. Differences in perceivable color can be analytically classified as: none 0-0.7, lightly 0.7-2.5, remarkable 2.5-3.0, appreciable 3.0-6.0, considerable 6.0-12.0, and biggest 12.0 (Ramirez-Navas & Rodriguez de Stouvenel, 2012).

Considering this parameter, from the results shown in *Table XIV*, there are significant color differences (ΔE) between the fortified cheeses, confirming the possibility of the visual perception of the human eye.

Cheeses C and E showed a considerable ΔE (6.0-12.0) while the cheeses G and F, enriched more than these, were found to be highly perceived (ΔE > 12) for all the period of storage.

Table XIV: Surface color parameters of fresh cheeses along the storage period.

CIELab	Cheese	T1	T2	T3	T4	T5
L*	**A**	$91.02^{A} \pm 0.97$	$90.61^{A} \pm 1.74$	$92.53^{A} \pm 4.98$	$91.28^{A} \pm 1.86$	$93.59^{A} \pm 0.97$
	C	$80.21^{C} \pm 1.25$	$81.13^{D} \pm 1.43$	$82.33^{C} \pm 1.15$	$80.51^{D} \pm 3.33$	$82.21^{C} \pm 0.93$
	G	$73.07^{E} \pm 2.44$	$73.83^{E} \pm 3.52$	$76.13^{E} \pm 1.84$	$74.17^{E} \pm 2.51$	$74.96^{E} \pm 3.42$
	E	$83.05^{B} \pm 1.62$	$84.87^{B} \pm 1.28$	$85.32^{B} \pm 1.01$	$85.12^{B} \pm 1.77$	$84.94^{B} \pm 2.16$
	F	$79.74^{D} \pm 2.64$	$81.51^{C} \pm 1.16$	$80.70^{D} \pm 1.55$	$81.20^{C} \pm 0.97$	$81.85^{D} \pm 1.72$
a*	**A**	$-0.95^{E} \pm 0.10$	$-1.03^{E} \pm 0.25$	$-0.85^{E} \pm 0.19$	$-0.53^{D} \pm 0.20$	$-0.63^{C} \pm 0.12$
	C	$-0.07^{B} \pm 0.16$	$-0.08^{B} \pm 0.15$	$-0.03^{B} \pm 0.13$	$-0.03^{B} \pm 0.21$	$-0.21^{B} \pm 0.18$
	G	$0.46^{A} \pm 0.33$	$0.46^{A} \pm 0.34$	$0.45^{A} \pm 0.34$	$0.39^{A} \pm 0.20$	$0.27^{A} \pm 0.26$
	E	$-0.64^{D} \pm 0.18$	$-0.78^{D} \pm 0.20$	$-0.67^{D} \pm 0.17$	$-0.66^{E} \pm 0.17$	$-0.67^{D} \pm 0.17$
	F	$-0.52^{C} \pm 0.19$	$-0.71^{C} \pm 0.17$	$-0.55^{C} \pm 0.13$	$-0.50^{C} \pm 0.24$	$-0.72^{E} \pm 0.16$
b*	**A**	$13.42^{E} \pm 0.68$	$13.76^{E} \pm 0.721$	$12.17^{E} \pm 0.85$	$14.14^{E} \pm 1.81$	$13.38^{E} \pm 0.77$
	C	$16.51^{D} \pm 0.78$	$17.31^{D} \pm 0.610$	$17.96^{D} \pm 0.76$	$17.99^{D} \pm 1.51$	$16.93^{D} \pm 0.84$
	G	$19.09^{C} \pm 1.08$	$19.64^{C} \pm 1.250$	$19.94^{C} \pm 1.83$	$19.42^{C} \pm 0.47$	$19.47^{C} \pm 0.56$
	E	$21.57^{B} \pm 0.878$	$21.22^{B} \pm 0.981$	$20.21^{B} \pm 1.73$	$21.87^{B} \pm 1.26$	$21.46^{B} \pm 0.92$
	F	$24.62^{A} \pm 1.99$	$24.88^{A} \pm 1.477$	$25.03^{A} \pm 1.27$	$25.99^{A} \pm 2.40$	$25.3^{A} \pm 1.09$
ΔE	**A**	-	-	-	-	-
	C	11.28^{A}	11.30^{B}	11.76^{B}	11.45^{B}	11.67^{B}
	G	18.88^{C}	17.84^{D}	18.19^{D}	17.93^{D}	18.98^{D}
	E	11.40^{A}	10.50^{A}	10.80^{A}	10.69^{A}	10.57^{A}
	F	15.90^{B}	15.97^{C}	16.06^{C}	15.56^{C}	16.03^{C}

Different letters indicate significant differences ($p < 0.05$) according to Tukey´s test. Cheeses C and G contain 0.5 and 1% of rosemary powder respectively; cheeses E and F were added with 0.25 and 0.5% of rosemary hydroethanolic extract, respectively. 1, 2, 3, 4 and 5 corresponding to storage days 1, 5, 8, 12 and 16, respectively.

I.3. Total phenolic content as affected by rosemary enrichment

The mean values of total phenolic content TPC (in mg gallic acid/g of cheese) of cheese samples are illustrated in Figure 10.

Overall, the fortified cheeses evidenced significantly higher ($P<0.05$) TPC values when compared to the non-enriched one throughout storage period, and these TPC values were increased with the concentration of rosemary added.

Cheese F was significantly different from the rest, presenting the highest TPC values (154 mg GAE/100g) followed by the cheese G (129 mg GAE/100g), while cheese C showed the lowest values (84 mg GAE/100g), noting that cheeses C and F were enriched with 0.5% of rosemary powder and ethanolic extract, respectively. Our results were in agreement with those obtained by Hala, Ebtisam, Sanaa, Badran, Marwa, and Said (2010) and (Ahmed Saad Gad & El-Salam, 2010)who observed the enrichment in TPC of fortified dairy products. This increasing could be ascribed to the richness of rosemary leaves in phenolic compounds identified as phenolic diterpenes, such as carnosic acid, carnosol, rosmanol, epi and iso-rosmanol, rosmadial and methyl carnosate, rosmarinic acid, caffeic acid and flavonoids (Abramovič, et al., 2012; Borrás-Linares, et al., 2015; Lemos, Lemos, Pacheco, Endringer, & Scherer, 2015; Shan, Cai, Sun, & Corke, 2005; Vallverdú-Queralt, Regueiro, Martínez-Huélamo, Rinaldi Alvarenga, Leal, & Lamuela-Raventos, 2014).

However, along storage period, it was noticed that the TPC decreased for control cheese from T1 to T5, for cheeses C, G and E from T3 to T5 and for cheese F from T4 to T5. According to Hala, M.F. El-Din (2010), this may be due to the transformation of phenolic compounds which highly unstable compounds and undergo numerous enzymatic and chemical reactions during food storage. After 16 days of storage, fortified cheeses exhibited more TPC than control by 102 and 74 mg GAE/100g for cheeses F and G containing 0.5% and 1% of rosemary ethanolic extract and powder, respectively.

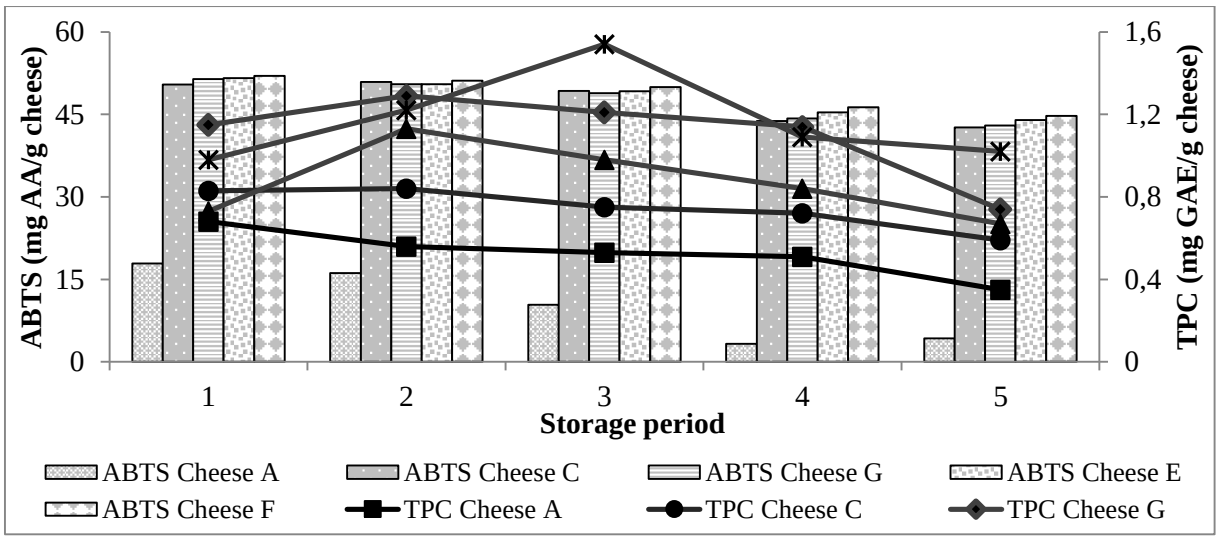


Figure 10. Total phenolic content and antioxidant activity of cheeses as affected by rosemary addition during storage. *Cheeses C and G contain 0.5 and 1% of rosemary powder respectively; cheeses E and F were added with 0.25 and 0.5% of rosemary hydroethanolic extract, respectively. 1, 2, 3, 4 and 5 corresponding to storage days 1, 5, 8, 12 and 16, respectively.*

In order to have additional insight on the effects of rosemary leaves supplementation, the major phenolic compounds were monitored along the storage time and results are summarized in table XV. The most prominent compounds in the fortified samples were the phenolic diterpenes [carnosol (C), carnosic acid (CA), 1, 2, methoxy-carnosic acid (MCA)] and the hydroxycinnamic acid [rosmarinic acid (RA)], while other phenolics, such as rosmanol (R) and the flavonoids kaempferol and luteolin, appeared as minor compounds. Consistent with the results from TPC, for the same level of supplementation (eg. cheese C vs cheese F), the extract produced a superior impact in the levels of these specific phenolic compounds. It is however important to mention that some fluctuations in the values observed along storage time may not only be due to phenolic degradations/rearrangements, but also result from a non-homogenous distribution of the powder/extract in the cheeses.

I.4. Antioxidant capacity

The ability of cheese samples to scavenge free radicals of ABTS was used as a criterion of their antioxidant activity. Results illustrated in Figure 10 indicated that cheese F exhibited slightly higher antioxidant capacity than the other cheese samples and supplemented rosemary cheeses

Table XV. Concentration of individual phenolic compounds (in mg/100g of cheese) of fresh cheeses along the storage period

	Cheeses	T1	T2	T3	T4	T5
RA	**A**	-	-	-	-	-
	C	2.82±1.89^{d}	1.65±1.87^{d}	2.44±4.17^{d}	1.92±3.19^{d}	1.93±6.26^{d}
	G	8.88±1.2^{b}	9.83±1.12^{b}	3.52±5.21^{c}	4.36±7.48^{c}	6.53±9.69^{c}
	E	7.79±5.58^{c}	6.88±0.82^{c}	10.12±7.03^{b}	8.20±9.79^{b}	9.80±2.51^{b}
	F	17.77±2.60^{a}	26.79±3.60^{a}	27.09±8.27^{a}	26.72±3.21^{a}	13.12±5.13^{a}
R	**A**	-	-	-	-	-
	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	G	0.82±2.53^{b}	1.64±4.26^{b}	2.14±3.60^{b}	1.89±1.15^{b}	2.21±4.3^{a}
	E	<LOQ	<LOQ	<LOQ	0.35±0.35^{c}	<LOQ
	F	3.12±4.63^{a}	3.64±1.48^{a}	4.49±2.53^{a}	5.41±5.13^{a}	1.80±1.50^{b}
C	**A**	-	-	-	-	-
	C	195.18±4.31^{c}	125.17±1.22^{d}	260.02±1.79^{c}	184.78±1.23^{c}	221.75±1.39^{c}
	G	416.11±2.75^{a}	376.84±3.29^{b}	395.88±4.07^{b}	324.89±2.54^{b}	418.45±3.41^{a}
	E	136.29±0.04^{d}	143.85±1.78^{c}	220.87±7.94^{d}	148.52±1.67^{d}	197.18±1.68^{d}
	F	289.88±1.93^{b}	476.29±9.90^{a}	511.47±1.46^{a}	546.80±2.90^{a}	255.55±9.76^{b}
CA	**A**	-	-	-	-	-
	C	80.39±5.82^{c}	67.10±7.62^{d}	93.11±1.39^{d}	68.36±4.13^{c}	88.22±6.58^{d}
	G	177.95±1.36^{a}	271.21±2.39^{a}	115.85±1.14^{c}	130.75±9.39^{b}	204.01±1.56^{a}
	E	132.79±1.01^{b}	124.93±3.97^{c}	156.37±6.27^{b}	130.01±1.35^{b}	167.68±7.48^{b}
	F	175.51±1.39^{a}	203.38±1.49^{b}	304.33±4.21^{a}	251.84±1.97^{a}	140.01±1.15^{c}
MCA	**A**	-	-	-	-	-
	C	57.06±6.82^{c}	34.62±2.5^{d}	60.72±1.17^{c}	43.01±2.24^{c}	52.28±2.05^{c} 80.26±4.11^{a}
	G	86.26±8.08^{a}	92.81±7.26^{a}	66.08±6.46^{b}	61.09±4.37^{b}	59.05±5.37^{b}
	E	40.83±2.28^{d}	45.59±0.8^{c}	58.46±2.23^{d}	41.71±3.10^{c}	52.70±1.21^{c}
	F	60.79±7.96^{b}	86.46±3.16^{b}	97.89±1.12^{a}	107.92±6.95^{a}	
Total	**A**	-	-	-	-	-
	C	335.45^{c}	228.54^{d}	416.29^{d}	298.07^{d}	364.18^{d}
	G	688.96^{a}	752.33^{b}	583.47^{b}	522.98^{b}	711.46^{a}
	E	317.70^{d}	321.25^{c}	445.82^{c}	328.79^{c}	433.71^{c}
	F	547.07^{b}	796.56^{a}	945.27^{a}	938.69^{a}	463.18^{b}

The phenolics were quantified from the areas of their peaks using external standard calibration curves. Abbreviations: RA: rosmarinic acid. R: rosmanol. C: carnosol. CA: carnosic acid. MCA: 1. 2.methoxy-carnosic acid. <LOQ : below limit of quantification. Cheeses C and G contain 0.5 and 1% of rosemary powder respectively; cheeses E and F were added with 0.25 and 0.5% of rosemary hydroethanolic extract. respectively. T1. T2. T3. T4 and T5 correspond to 1. 5. 8. 12 and 16 days of storage. respectively. Different letters indicate significant differences ($p < 0.05$) according to Tukey´s test.

showed a significant (P> 0.05) higher antioxidant potential compared to the control during all storage period. This could partly be due to the presence of rosemary diterpenoids in these cheeses. According to Loussouarn, Krieger-Liszkay, Svilar, Bily, Birtić, and Havaux (2017), carnosic acid and carnosol are both potent antioxidants. In addition, several studies have reported that the presence of phenolics of plant materials in enriched food products could be associated with high antioxidant capacity (Apostolidis, Kwon, & Shetty, 2007; Branciari, nucci, Trabalza-Marinucci, Codini, Orru, Ortenzi, et al., 2015; Felfoul, Borchani, Samet-Bali, Attia, & Ayadi, 2017; Hala, Ebtisam, Sanaa, Badran, Marwa, & Said, 2010; Muniandy, Shori, & Baba, 2016; Ribeiro-Santos, et al., 2015). In the other hand, the fluctuations in the fluctuations in values of ABTS of all rosemary cheeses remained insignificant (P > 0.05) along the storage period. However, it was noteworthy that the percentage of ABTS gradually decreased throughout storage period for all cheese samples.

II. Microbiological Analysis

In this section, we looked at the effect of adding rosemary (powder or extract) on lactic acid bacteria. Thus, monitoring of the lactobacilli and lactococci load was carried out for the different cheeses during their storage period.

An absence of total coliforms, yeasts and molds was recorded in the various cheese samples analyzed. This indicates compliance with hygienic measures during their transport and their enrichment in rosemary.

The level of lactic flora in cheese samples is illustrated in figure 11. The obtained results showed that at T1 the level of lactic flora in cheese A (control) was $7.1.10^8$ CFU/g for lactobacilli and $6.2.10^8$ CFU/g for lactococci. An increase was observed until reaching a load of $8.9.10^8$ CFU/g for lactobacilli (T2) and a maximum load of $8.6.10^8$ CFU/g for lactococci (T3). After that, a decrease in the level of lactic acid bacteria has been noted during storage.

Otherwise, an increase in the percentages of rosemary powder or extract in cheese samples induced a decrease in the bacterial number where the rate does not exceed 10^6 CFU/g at from

T4. It was noticed a significant (P> 0.05) difference between all the cheeses during the whole period of their storage.

According to Ray B. (1996), no regulation recommends a specific actic for a load for fresh cheese, the loss of viability of the lactic flora during refrigeration is usually scaled down. However, cheeses supplemented with rosemary ethanolic extract contain less lactic flora load than those supplemented with powder.

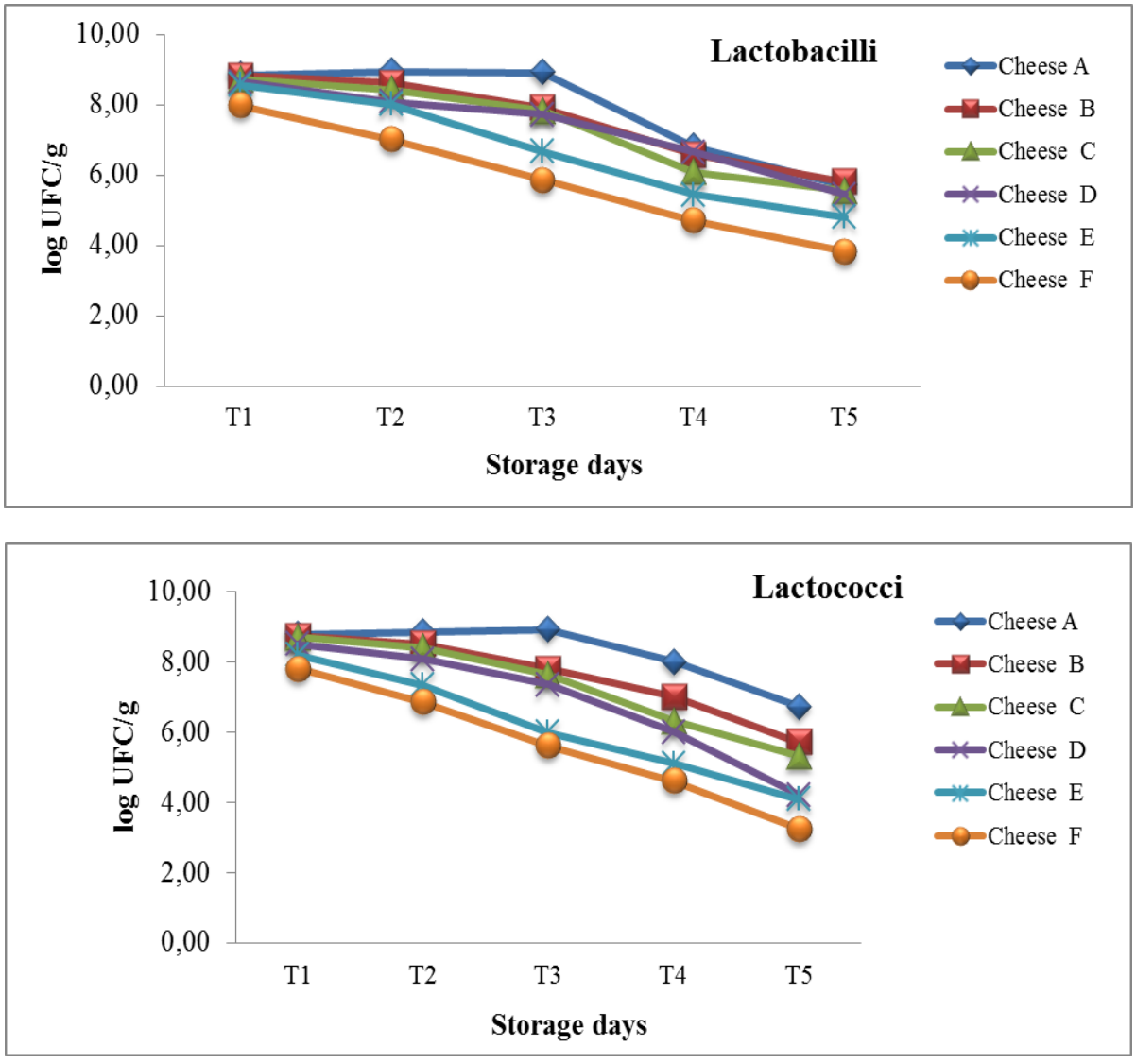


Figure 11: Evolution of the lactic flora of cheese samples during storage

Cheeses B, C and D contain 0.25, 0.5 and 0.75% of rosemary powder respectively; cheeses E and F were added with 0.25 and 0.5% of rosemary hydroethanolic extract, respectively. T1. T2. T3. T4 and T5 correspond to 1. 5. 10, 14 and 18 days of storage, respectively.

III. Sensory analysis (hedonic test)

The hedonic test results are illustrated in *Table XVI*. As represented in this table, the rosemary supplementation to cheese samples significantly ($p < 0.05$) influenced the sensorial characteristics.

Table XVI: Effect of rosemary supplementation on hedonic appreciation of cheese samples

		Sensory parameters					
		Color	***Odor***	***Acidity***	***Aroma***	***Aftertaste***	***Texture***
Control	Cheese A	1.38 ±0.88^{a}	2.18 ±1.02^{a}	3.38 ±0.65^{b}	1.43 ±1.22^{a}	2.07 ±0.97^{a}	3.42 ±1.02^{b}
Rosemary powder concentrations	**0.25%** (Cheese B)	2.38 ±1.15b^{c}	2.98 ±0.89^{a}	3.25 ±1.21^{b}	3.20 ±1.17^{b}	2.88±1.18^{bc}	3.25 ±1.32^{a}
	0.50% (Cheese C)	2.63 ±0.98^{cd}	4.13 ±0.95^{b}	3.10 ±0.88^{ab}	3.63 ±1.11^{c}	3.25±1.31^{c}	3.38 ±1.16^{ab}
	0.75% (Cheese D)	2.85 ±0.78^{d}	4.52 ±1.11^{b}	2.75 ±0.94^{a}	4.13 ±0.88^{d}	3.88±0.88^{d}	3.25 ±1.15^{a}
Rosemary extract concentrations	**0.25%** (Cheese E)	2.15 ±0.97^{b}	3.04 ±1.21^{a}	3.15 ±1.16^{b}	3.07 ±0.98^{b}	2.45 ±0.89^{b}	3.36 ±1.31^{ab}
	0.50% (Cheese F)	2.75 ±0.89^{d}	4.05 ±0.95^{b}	3.07 ±0.88^{ab}	3.45 ±0.97^{c}	3.07 ±1.07^{bc}	3.26 ±0.94^{ab}

Different letters indicate significant differences (p <0.05) according to Tukey´s test.

Except the control cheese, which was of a white color, all the other cheeses had beige to yellow-green color. Significant differences were observed between all cheeses and the highest color scores were detected in cheeses D and F, with high percentages of rosemary powder and ethanolic extract, respectively. The rosemary supplementation hided the lactic odor of cheeses, and those supplemented with 0.5 % and 0.75 % of plant extracts were significantly highly scored compared to the other cheeses. Regarding the acid taste of cheeses, the panelists evaluated them as moderately acid. This could be due to the non-significant differences between the pH values of cheeses. An absence of rosemary aroma together with aftertaste was detected Cin the control cheese but these sensory attributes were considered superiors and highly

significantly perceptibles by the panelists for other cheeses. Cheese D was the most scored one. The smooth-texture of all the cheeses was judged to be almost average and scored from 3.25 to 3.42. So, it could be concluded that the addition of rosemary did not significantly influenced the texture of the cheeses.

Among the main objectives of this study, is to provide a distinctive flavor and aroma following cheeses rosemary supplementation and to provide too a flavor enhancement for a better acceptability by the consumers. Overall preferences (acceptability) were significantly ($p < 0.05$) highly scored for cheese C (6/9) followed by cheese D (5.7/9), then cheese F (5.3/9). As showed in Figure 12, cheeses C, D and F were most preferred by the panelists (60-80 %) and their score results were higher than those of cheese B (40-60 %), cheese E and cheese A taken as control (20-40 %).

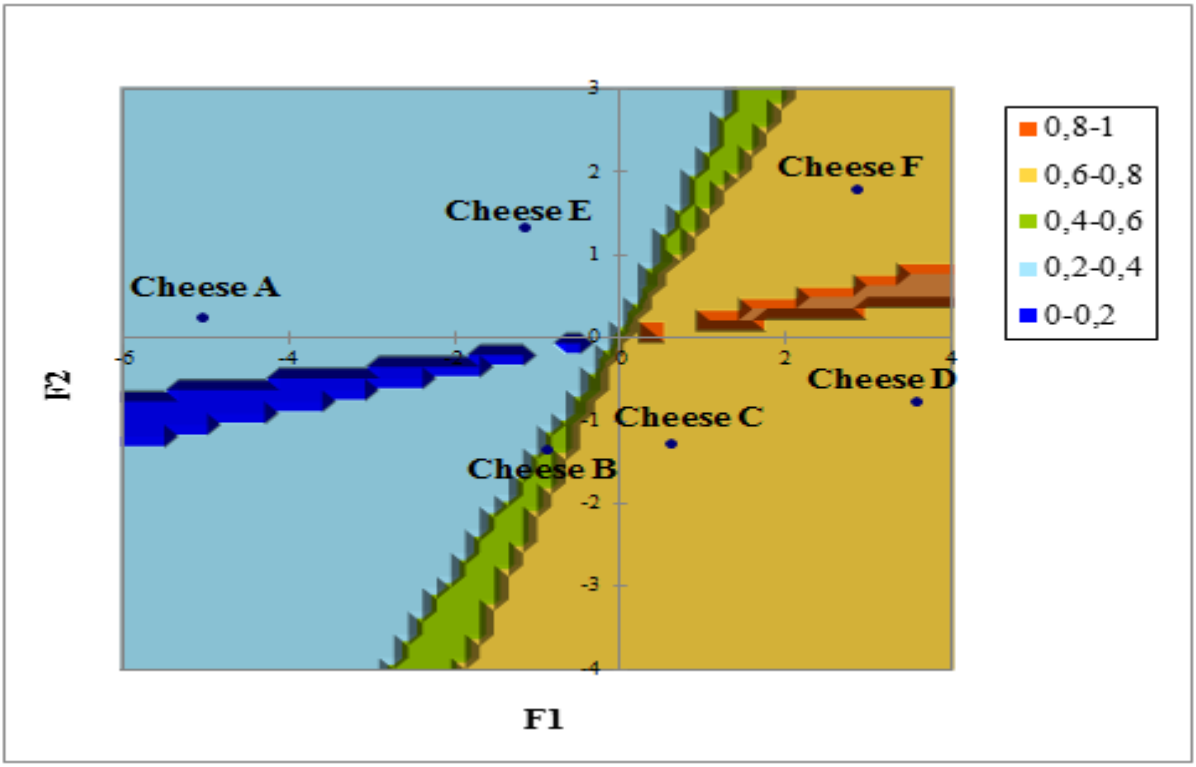


Figure 12: Preference mapping of panelists

Cheese A: control, rosemary powder added to Cheese B: 0.25%, Cheese C: 0.5% and Cheese D: 0.75%, ethanolic extract added to Cheese E: 0.25% and Cheese F: 0.5%

IV. Relationship between physicochemical parameters, sensory attributes, phenolics and antioxidant capacity of cheese samples

In order to better understand the interactions between the physicochemical parameters and the sensory characteristics, the implementation of the PCA could be an interesting solution. The obtained PCA model explained 87.68 % of variability found in the analyzed data: Component 1 (PC1, Figure 13) of the PCA accounted for 73.91 % of the variance and was expressed towards its positive pole by antioxidant activity (AA), polyphenols (PP), color (Co), odor (Od), aroma (Ar) and aftertaste (Af), which showed good correlations between them. While component 2 (PC2, Figure 13), which included proteins (Pr) towards its positive pole, and fat (Fat) towards its negative pole, accounted for 13.78 % of the variance.

The results of the PCA (Figure 13) highlighted a significant correlation (r = 0.845) between phenolic compounds (PP) and sensory attributes as color (Co, r = 0.886), odor (Od, r = 0.953), aroma (Ar, r = 0.857) and aftertaste (Af, r = 0.867), similar results were reported by Lund, Nicolau, Gardner, and Kilmartin (2009) and Guo, Yue, and Yuan (2019). Increased concentrations of the phenolic compounds significantly affected the headspace concentration of most aroma compounds, and each polyphenol had a unique effect when combined with a specific aroma compound, either, suppressing, accentuating or showing little effect on the perception of the aroma compounds. A study on the relationship between total polyphenols and color, Hodžić, Karahmetović, Saletović, and Šestan (2007) concluded that color values of the wort and beer increased with polyphenol content increase.

Through Figure 13**.A**, it can be also seen that pH was correlated with acidity taste (Ac, r = 0.713). According to da Conceicao Neta, Johanningsmeier, Drake, and McFeeters (2007),sour taste intensity was linearly related to the molar concentration of hydrogen ions and all organic acid species that had at least 1 protonated carboxyl group. All of the organic acids in each of their protonated forms were equivalent in their ability to cause a sour taste.

To explore the possible relationship between surface color attributes (L*a*b*) of cheese samples, their identified phenolic compounds (RA, R, C, CA, MCA) and their antioxidant capacity (ABTS), the principal component analysis (PCA) was used. Data illustrated in figure 13.B revealed that the PCA model explained 87.39% of variability found in the analyzed data, with respectively 67.70% of the variability explained by the first principal components (PC1) and 19.69% by the second principal components (PC2). PC1 was defined by ABTS (r^2 = 0.812), phenolic compounds [MCA (r^2 = 0.972), C (r^2 = 0.962), CA (r^2 = 0.945), RA (r^2 = 0.812), R (r^2 = 0.723)], and coordinates b* (r^2 = 0.827) and L* (r^2 = -0.798), while thecoordinate a* (r^2 = 0.849) was the largest contributor to the second principal components (PC2).

The biplot obtained from PCA (Figure 13) showed a positive correlation between ABTS content and phenolic compounds, mainly MCA (r^2 = 0.805), CA (r^2 = 0.726) and C (r^2 = 0.697); this correlation suggest that these phenolics are the strongest contributors to the radical scavenging capacity in the fortified cheese samples. Furthermore, a good correlation was observed between the attribute b* (r^2 = 0.635 to 0.849), ABTS and the identified polyphenols; the attribute a* was moderately correlated (r^2 = 0.439 to 0.476) with them while, the attribute L* was correlated negatively. So, it could be noticed that the attribute L* is closer to cheeses E and A while the attributes a* and b* are closer to fortified cheeses than the control (Cheese A).

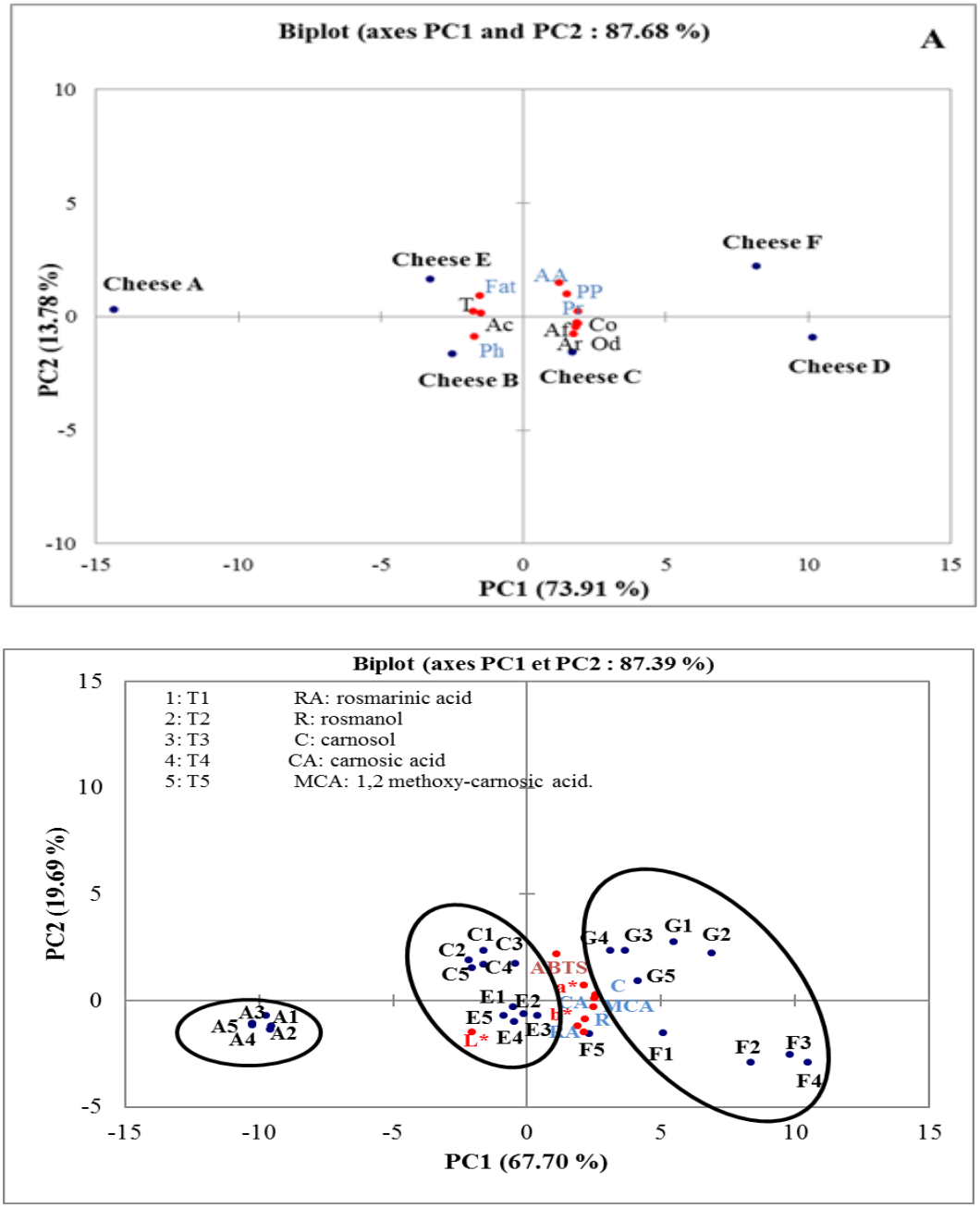


Figure 13*:** Relationship between physicochemical parameters, sensory attributes phenolics and antioxidant capacity of cheese samples. **A*: Biplot illustrating the relationship between physicochemical parameters, phenolics and sensory attributes of cheese samples-**B:** Biplot illustrating the relationship between color, ABTS and rosemary-cheese polyphenols.

PP: phenolic compounds, Pr: proteins, Co: color, Od: odour, Ac: acidity taste, Ar: aroma, Af: aftertaste, T: smooth-texture, Cheese A: control, rosemary powder added to Cheese B: 0.25%, Cheese C: 0.5% and Cheese D: 0.75%, ethanolic extract added to Cheese E: 0.25% and Cheese F: 0.5%. RA: rosmarinic acid, R: rosmanol, C: carnosol, CA: carnosic acid, MCA: 1, 2 methoxy-carnosic acid. Cheeses C and G contain 0.5 and 1% of rosemary powder respectively; cheeses E and F supplemented with 0.25 and 0.5% of rosemary hydroethanolic extract, respectively. A, B, C, D, E with indices 1, 2, 3, 4 and 5 indicate cheeses A, C, G, E, F during storage days 1, 5, 8, 12 and 16, respectively.

Through Figure 13, it permitted a neat separation of control cheese and experimental cheeses fortified with rosemary as well as an individualization of three different groups of cheeses. Group I: of cheeses (G, F) containing very high rosemary content with a positive correlation; Group II: consisting of cheeses (C, E) with a low rosemary content with an intermediate correlation and Group III: containing control cheese with a negative correlation. This group distinction showed that cheeses (G, F) with high rosemary content (powder / extract) were the richest in polyphenols with a strong antioxidant capacity.

THIRD PART:

Enrichment of whey milk by rosemary

Chapter I:

MATERIALS AND METHODS

I. Whey milk-rosemary and whey milk proteins content

This test was carried out in order to know the influence of rosemary on the protein composition of milk (whey).

I.1. Samples preparation

A branch of 5 g of rosemary was added to 100 ml of fresh cow's milk, a control was prepared with 100 ml of milk. The samples were incubated at room temperature for 24 h. All the analyses in this part are carried out on the whey filtrate (centrifugation 30min / 4000tr, filtration of the supernatant).

I.2. Protein content

The proteins concentration of samples was quantified by the Bradford method using bovine serum albumin as a standard; results are expressed as µg equivalent BSA per mL of whey: 5 mL of Bradford's reagent are added to 100 mL of the sample (whey or whey-rosemary) the mixture is vortexed and then incubated for 1 hour in the dark at room temperature. The absorbance measurement was performed at 595 nm.

II. Turbidity test

Polyphenols are known for their interactional characteristic with proteins. Polyphenol-protein interactions can be visualized by the turbid appearance of the solution (Simon, 2003). Monitoring of turbidity was done by spectrophotometry by measuring the optical density of the solution at the wavelength where the optical density is in the maximum.

The measurement of the optical density was carried out in terms of the concentration of rosemary extract or caffeic acid. For that, 1.5 mL of the milk whey filtrate was mixed with 1.5 mL of rosemary extract (0.12 to 3 mg of methanolic rosemary extract / mL of methanol) or caffeic acid (0.05 to 1 mg caffeic acid / mL of methanol). After 1 hour of incubation at 37 ° C, the absorbance was achieved at 420 nm and 600 nm.

III. Determination of phenolic compounds in milk – rosemary samples

To determine the content of phenolic compounds in milk-rosemary samples, the colorimetric method of Folin-Ciocalteu was adopted according to the method of Velioglu et al. (1998): 1.5ml folin (1/10) are added to 200µL of the whey-rosemary solution, after 5min in the dark, 1.5mL NaCO3 (7.5%) are added to the mixture. After 30 min incubation in the dark, the absorbance is read at 750nm. Gallic acid was used as standard and TPC concentration was expressed in mg of Gallic Acid Equivalents (GAE) per g of fresh cheese. The blank was prepared with the filtrate of the whey from the milk.

Chapter II:

RESULTS AND

DISCUSSION

I. Determination of protein in milk whey

This dosage is carried out in order to know the influence of rosemary on the protein composition of milk (whey). The protein assay was performed on milk whey and on milk whey - rosemary.

Table XVII: Proteins concentration in whey-rosemary

	Whey milk	**Whey-rosemary**
Protein concentration (µg EBSA/mL whey)	0,96 ± 0,08	0,89 ±0,11

The protein content in the samples tested is very low; however a small difference is recorded for these two solutions. This difference may be due to the complexation of these compounds with the polyphenols of rosemary.

II. Turbidity test

The turbidity test was carried out on two different solutions, namely the solution of the ethanolic extract of rosemary and that prepared with caffeic acid, which is a phenolic acid representative of *Rosmarinus officinalis*.

This test makes it possible to follow the evolution of the polyphenol-protein complexes by spectrophotometry at a wavelength of 420 nm by considering that at this wavelength, the intensity of the absorption of light is proportional to the concentration of complexes formed. The measurement of the turbidity was also carried out at 600 nm according to the method of Abi azar (2007).

II.1. Turbidity test of the rosemary extract-whey proteins mixture

As shown in figure 14, the filtrate of the whey from the milk during this experiment remains clear; however a turbid appearance is developed by the addition of *Rosmarinus officinalis* extract, indicating the formation of protein-polyphenol complexes.

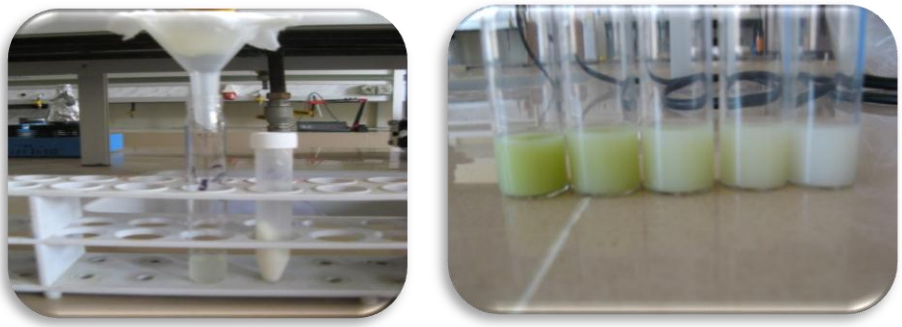


Figure 14: Effect of rosemary extract concentrations on whey

Figure 14 shows changes in absorbance at the two measurement wavelengths (420 and 600 nm) depending on rosemary extract concentrations.

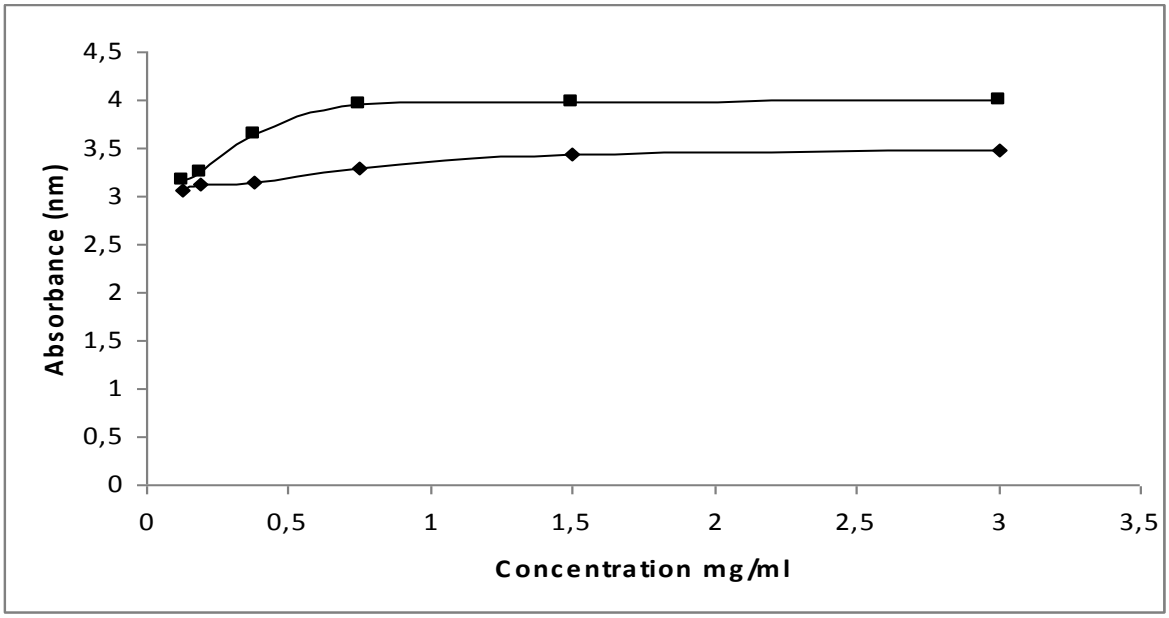


Figure 15: Variation in optical density depending on the concentration of *Rosmarinus officinalis* extract. (■) 400 nm, (♦) 600 nm.

From these results, it can be seen that the evolution of the optical density of the complexes formed is different at the two measurement wavelengths (420 and 600 nm). It is clear that the optical density of the complex at 420 nm increases with the concentration of the extract of *Rosmarinus officinalis* and this for the first three concentrations tested (3.65; 3.25; 3.17 mg / mL), plus the concentration the more the turbidity increases up to a maximum value of the optical density (3.9) which remains practically unchanged.

This can be explained by the saturation of the proteins present in the milk whey by polyphenols, the concentration of which is quite high. When the number of binding sites equals those of the protein, a large network of polyphenol – protein complexes is formed, indicating saturation of all binding sites.

The optical density of the complexes at 600 nm remains unchanged for all the concentrations tested. This result does not agree with that obtained by Abi azar. (2007), who found the formation of complexes whose optical density is proportional to the concentration of the carob extract. This difference can be explained by the fact that the polyphenols of carob (gallotannins) are different from those of rosemary (phenolic acids) and the whey protein-gallotannins complexes are detectable at 600 nm while those obtained with caffeic acid are detectable at 420 nm.

II.2. Turbidity test of the caffeic acid-whey protein mixture

The whey filtrates in the presence of caffeic acid developed a turbid appearance, which can be interpreted as the complexation of caffeic acid with protein.

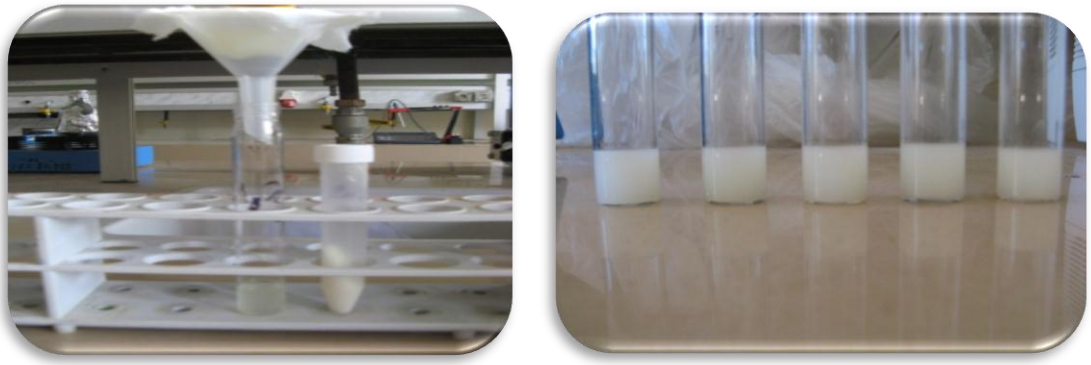


Figure 16: Effect of varying the concentration of caffeic acid on protein complexation.

In the case of caffeic acid, there is also a large difference in the optical density of the complexes formed at the two readings 420 and 600 nm. At 600 nm no change in the optical density was recorded for all the concentrations tested.

On the other hand, this optical density becomes proportional to the concentration of caffeic acid at 420 nm and this for the first two (2.22 mg / mL; 1.92 mg / mL) concentrations tested, and then it stabilizes from 0, 2 mg / mL. These results are similar to those obtained with the rosemary extract. Therefore, we can suggest that indeed it is the caffeic acid present in our extract that contributed to the formation of the complexes.

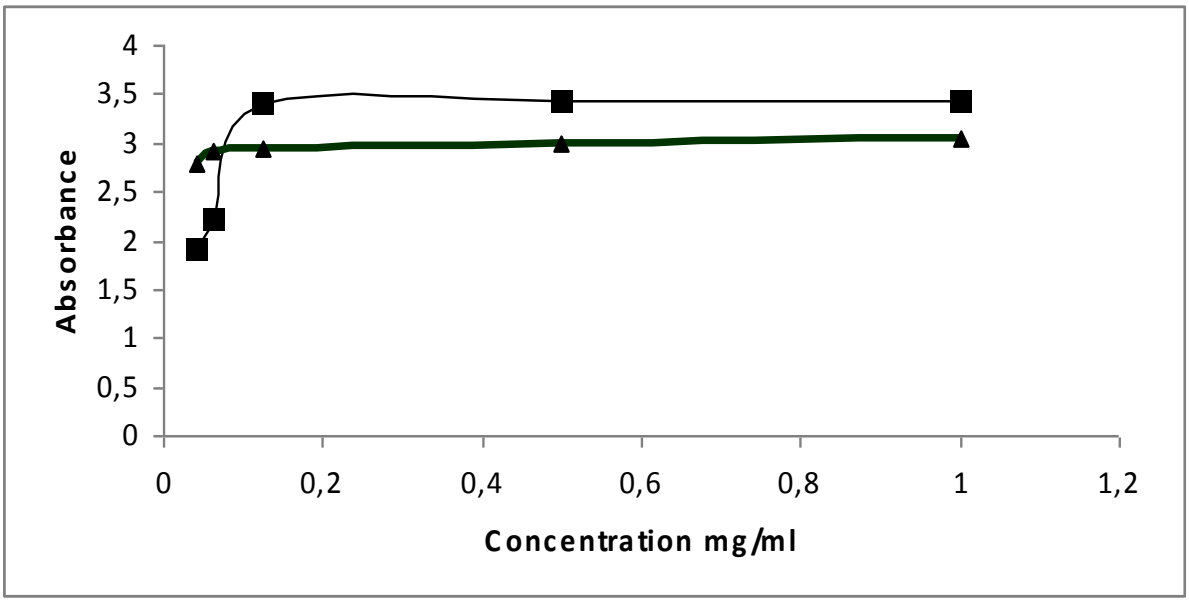


Figure17: Variation of the optical density as a function of the concentration of caffeic acid.

(■) 400 nm, (▲) 600 nm.

It has been suggested that the nature and structure of polyphenols affect access to acceptor sites during interactions with proteins (Siebert *et al.*, 1996). Monomers, dimers and some oligomers cannot form enough cross-bridges and strong complexes with proteins (Jones *et al.*, 1976). This suggestion is not verified in our case, because we obtained a complexation with caffeic acid, which leads us to confirm that it is indeed the caffeic acid, which caused the formation of the complexes after adding the rosemary branch with whey.

III. Determination of total polyphenols in the whey filtrate of mik

The concentration of polyphenols in the whey filtrate is 0.035 ± 0.01 mg EAG / mL. This value is very low; it may also relate to the presence of reducing sugars in the whey and not to the presence of phenolic compounds. From this result it can be concluded that cow's milk is not a

medium which extracts polyphenols from rosemary. Several hypotheses can be proposed, either the polyphenols of rosemary are complexed with the proteins of the whey (this method does not allow the determination of the polyphenols) or else with the insoluble proteins of the milk and in this case they are passed in the precipitate.

General conclusion and perspectives

In this study, rosemary (*Rosmarinus officinalis*) leaves were incorporated in fermented milk and the impact on physico-chemical changes occurring in freshcheese during 18 days storage period and possible effects on the phytochemistry of the plant were examined.

Microwave-Assisted Extraction (MAE) of *Rosmarinus officinalis* L. leaves was optimized through the response surface methodology (RSM) approach. The optimal conditions were equal to 78.162 %, 351.825 W, 101.623:1, and 122.648 s, for ethanol concentration, microwave power, solvent to raw material ratio, and extraction time, respectively. It can be concluded that rosemary leaves can be used as a good source of valuable compounds since it was possible to obtain up to 15.01 % (on dry weight basis) of total phenolic compounds (TPC).UHPLC-DAD-ESI-MS^n analysis revealed the major presence of rosemarinic acid (RA), rosmanol (R), carnosic acid (CA), carnosol (C) and methyl carnosic acid (MCA) in rosemary extract which had powerful antioxidant and antidiabetic effects comparable to those of tested standards.

Fresh cheese was supplemented with powder (cheese B: 0.25 %, cheese C: 0.5 %, cheese D: 0.75 %, G: 1%), and ethanolic extract (cheese E: 0.25 %, cheese F: 0.5 %) of *Rosmarinus officinalis* L. leaves. The main conclusions of this part of study are:

- Cheese physicochemical characteristics (pH, fat, proteins) were not influenced by rosemary addition. However, this fortification considerably affected cheese color parameters: with the increase of rosemary concentration, L* values decreased B* increased, demonstrating that fortified cheeses were darker, greener and yellower than non- fortified one;
- This supplementation allowed the obtention of a final product with improved phenolics content and antioxidant capacity when compared to the control (Cheese A).Rosemary powder/extract addition positively affected the phenolic content in cheese during storage period; cheese E2 had the highest amount of polyphenols and the antioxidant property followed by cheese P2. UHPLC-DAD-ESI-MS^n analysis revealed the presence of RA, R, CA, C and MCA in supplemented cheeses. Moreover, the use of the multivariate statistical analysis (PCA) confirmed the relationship between antioxidant activity and these phenolic compounds.

Additionally, an individualization of three different groups of cheeses was obtained with a neat separation of control cheese and experimental cheeses gradually fortified with rosemary;

- The influence of rosemary addition on cheese sensory characteristics was, especially, related to the intensity of their particular odor, aroma and after taste scores. It was concluded that cheeses C, D and F were most appreciated (60-80%) over the rest of cheeses when the effects on the antioxidant capacity and the sensory characteristics were jointly considered;
- The results obtained from the monitoring of Lactobacilli and Lactococci revealed that cheese samples contain high loads in the first week, a load bacterial which gradually decreases during storage period. The increase in the concentrations of rosemary powder/extract in cheese samples resulted in decrease of lactic flora during storage time.

In this study rosemary extract and whey protein complexation was highlighted by the turbidity test. It was concluded that rosemary phenolic acids were responsible of this complexation as complexes were detectable at 420nm.

Addition of rosemary leaves, in the form of extract or powder, in cheese formulation is strongly recommended as it exerts features valuable for human health and nutrition. A production of this cheese in an industrial-scale would be very interesting.

Further studies are needed to test this supplementation on other kinds of cheese then, assess the consumer sensory and test the bioavailability of rosemary polyphenols will be so advantageous. In addition, it will be more interesting to study the gastrointestinal stability of polyphenols in fortified cheeses and evaluate the antioxidant activity of taken samples during simulated digestion procedures.

Investigations on the feasibility of an industrial-scale continuous MAE process for the recovery of phenolic compounds from rosemary leaves will be needed. Besides, it is promising that these valuable extracts could be used for the extension of products shelf life, as dietary supplements as well as functional food ingredients.

REFERENCES

REFERENCES

- **Abayneh Kassahun and Gezu Feleke (2019).** Proximate analysis, physicochemical properties and chemical characterization of *Rosmarinus officinalis* L. oil. *Nat. Volatiles & Essent. Oils; 6(2): 20-24*.

- **Abi Azar R, (2007).** Complexation des protéines laitières par les extraits de gousses vertes de caroubier**,** Propriétés technologiques des coagulums obtenus. *Thèse De doctorat de AgroParisTech, 159p.*

- **Abramovič, H., Terpinc, P., Generalić, I., Skroza, D., Klančnik, A., Katalinić, V., & Smole Možina, S. (2012).** Antioxidant and antimicrobial activity of extracts obtained from rosemary (Rosmarinus officinalis) and vine (Vitis vinifera) leaves. *Croatian journal of food science and technology*, *4*(1), 1-8.

- **Aguilar F., D. Boskou, D. Gott, S. Grilli, R. Guertler, K. Hulshof, J.C. Larsen, J.C. Leblanc, C. Leclercq, A. Mortensen, D. Parent-Massin, I. Pratt, I. Rietjens, P. Tobback, G. Speijers, F. Toldra. (2008).** Use of rosemary extracts as a food additive–scientific opinion of the panel on food additives, flavourings, processing aids and materials in contact with food. *EFSA J. 721:1-29.*

- **Ahmad, S.T., Arjumand, W., Nafees, S., Seth, A., Ali, N., Rashid, S., Sultan, S., (2012).** Hesperidin alleviates acetaminophen induced toxicity in wistar rats by abrogation of oxidative stress, apoptosis and inflammation. *Toxicol. Lett. 208 (2), 149–161.*

- **Ali, M. S., Saleem, M., Ali, Z., & Ahmad, V. U. (2000).** Chemistry of Zataria multiora (Lamiaceae). *Phytochemistry, 55, 933e936*.

- **Ali, M.K.A., (2013).** Interactions of Food Proteins with Plant Phenolics – Modulation of Structural, *Techno and Bio-functional Properties of Proteins. Potsdam University (Ph.D. thesis).*

- **Almela Luis, Sanchez-Munoz Blas, Fernandez-Lopez Jose A., Roca Maria J., Rabe Virginia (2006).** Liquid chromatograpic–mass spectrometric analysis of phenolics and free

radical scavenging activity of rosemary extract from different raw material. *Journal of Chromatography A, 1120 (2006) 221–229. doi:10.1016/j.chroma.2006.02.056.*

- **Almela, L.; Sanchez-Munoz, B.; Fernandez-Lopez, J. A.; Roca, M. J. and Rabe, V. (2006).** Liquid chromatographic–mass spectrometric analysis of phenolics and free radical scavenging activity of rosemary extract from different raw material. *J. Chromatogr., 1120: 221-229.*

- **Alonso, J. (2004).** Treaty of Fitofarmacos and Nutraceuticos. *Barcelona: Corpus, p: 927-930.*

- **Al-Sereitia, M. R., Abu-Amerb, K. M., & Sena, P. (1999).** Pharmacology of rosemary (*Rosmarinus officinalis* L.) and its therapeutic potentials. *Indian Journal of Experimental Biology, 37, 124e131.*

- **Al-Sereitia, M. R.; Abu-Amerb, K. M. and Sena, P. (1999).** Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials. *Indian J. Exp. Biol., 37 (2): 124-131.*

- **Amini, A. (1997).** Illustrated Dictionary of Therapeutic Plants and their Traditional Usage in Kurdistan. *Taqbostan Publication, Khoramabad.*

- **Andrén, A. (2002).** Rennets and coagulants. In: Roginski, H., *et al.* (Eds.) *Encyclopaedia of Dairy Sciences.* London: Academic Press. p. 281-286.

- **Angioni, A., Barra, A., Cereti, E., Barile, D., Coisson, J. D., Arlorio, M., et al. (2004).** Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil of Rosmarinus officinalis L. *Journal of Agricultural and Food Chemistry, 52, 3530e3535.*

- **Apostolidis, E., Kwon, Y.-I., & Shetty, K. (2007).** Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. *Innovative Food Science & Emerging Technologies, 8*(1), 46-54.

- **Aqel, M. B. (1991).** Relaxant effect of the volatile oil of *Rosmarinus officinalis* on tracheal smooth muscle. *J. Ethnopharmacol., 33: 57-62.*

- **Arnold, N., Valentini, G., Bellomaria, B., & Hocine, L. (1997).** Comparative study of the essential oils from Rosmarinus eriocalyx Jordan & Fourr. from Algeria and R. officinalis L. from other countries. *Journal of Essential Oil Research, 9, 167e175.*

- **Arslan, D., & €Ozcan, M. Musa (2008).** Evaluation of drying methods with respect to drying kinetics, mineral content and colour characteristics of rosemary leaves. *Energy Conversion and Management, 49(5), 1258e1264.*

- **Arts, M.J., Haenen, G.R., Voss, H.P., Bast, A., (2001).** Masking of antioxidant capacity by the interaction of flavonoids with protein. *Food Chem. Toxicol. 39, 787–791.*

- **Atti-Santos, A. C.; Rossato, M.; Pauletti, G. F.; Rota, L. D.; Rech, J. C.; Pansera, M. R.; Agostini, F.; Atti-Serafini, L. and Moyna, P. (2005).** Physico-chemical evaluation of *Rosmarinus officinalis* L. essential oils. Braz. Arch. of Biol. and Tech., *Int. J., 48 (6): 1035-1039.*

- **Auldist, M., Johnston, K.A., White, N.J. & Fitzsimons, W.P. (2004).** A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Friesian and Jersey dairy cows. *Journal of Dairy Research* 71, 51-57.

- **Babu, U. S.; Wiesenfeld, P. L. and Jenkins, M. Y. (1999).** Effect of dietary rosemary extract on cell-mediated immunity of young rats. *Plant Food Hum. Nutr., 53(2):169-174.*

- **Bai, X. L., Yue, T. L., Yuan, Y. H., & Zhang, H. W. (2010).** Optimization of microwave-assisted extraction of polyphenols from apple pomace using response surface methodology and HPLC analysis. [Research Support, Non-U.S. Gov't Validation Study]. *J Sep Sci, 33*(23-24), 3751-3758. doi: 10.1002/jssc.201000430.

- **Bakirel, T., Bakirel, U., Keles¸ , O. U., Ulgen, S. G., & Yardibi, H. (2008).** In vivo assessment of antidiabetic and antioxidant activities of rosemary (Rosmarinus officinalis) in alloxan-diabetic rabbits. *Journal of Ethnopharmacology, 116(1), 64e73.*

- **Barbano, D.M., Rasmussen, R.R. & Lynch, J.M. (1991).** Influence of milk somatic-cell count and milk age on cheese yield. *Journal of Dairy Science* 74(2), 369-388.
- **Barbara Dawson (2020).** Rosemary Herb For Beginners and Dummies.
- **Bartolome, B., Estrella, I., Hernandez, M.T., (2000).** Interaction of low molecular weight phenolics with proteins (BSA). *J. Food Sci. 65, 617–621.*
- **Bauer, K.; Garbe, D. and Surburg, H. (1997).** Common Fragrance and Flavor Materials. *3rded. Germany: Wiley-VCH.*
- **Begum, A., Sandhya, S., Ali, S. S., Vinod, K. R., Reddy, S., & Banji, D. (2013).** An in-depth review on the medicinal flora *Rosmarinus officinalis* (Lamiaceae). *Acta Scientiarum Polonorum Technologia Alimentaria, 12(1), 61e73.*
- **Bibi Sadia1*, Shazia Irfan1 and Zahoor Ahmed Bazai (2016).** Chemical composition of rosemary (*Rosmarinus officinalis* L.) leaves under salt stress. *Pure Appl. Biol., 5(2): 355-360. http://dx.doi.org/10.19045/bspab.2016.50046.*
- **Birtić, S., Dussort, P., Pierre, F.-X., Bily, A. C., & Roller, M. (2015).** Carnosic acid. *Phytochemistry, 115*, 9-19.
- **Blumenthal, M.; Goldberg, A. and Brinkmann, J. (2000).** Herbal Medicine: Expanded Commission Monographs. *Boston (MA): Integrative Medicine Communications.*
- **Borras Linares I., Arraez-Roman D., Herrero M., Ibanez E., Segura-Carretero A., Fernandez-Gutierrez A. (2011).** Comparison of different extraction procedures for the comprehensive characterization of bioactive phenolic compounds in Rosmarinus officinalis by reversed-phase high-performance liquid chromatography with diode array detection coupled to electrospray time-of-flight mass spectrometry. *Journal of Chromatography A, 1218, 7682–7690.*
- **Borrás-Linares, I., Pérez-Sánchez, A., Lozano-Sánchez, J., Barrajón-Catalán, E., Arráez-Román, D., Cifuentes, A., Micol, V., & Carretero, A. S. (2015).** A bioguided

identification of the active compounds that contribute to the antiproliferative/cytotoxic effects of rosemary extract on colon cancer cells. *Food and Chemical Toxicology, 80,* 215-222.

- **Borrás-Linares, I., Stojanović, Z., Quirantes-Piné, R., Arráez-Román, D., Švarc-Gajić, J., Fernández-Gutiérrez, A., & Segura-Carretero, A. (2014).** Rosmarinus officinalis leaves as a natural source of bioactive compounds. *International journal of molec ular sciences, 15*(11), 20585-20606.
- **Bown, D. (1995).** Encyclopedia of Herbs and their Uses. *Dorling Kindersley, London.*
- **Bradley, P. R. (2006).** British Herbal Compendium: A Handbook of Scientific Information on Widely Used Plant Drugs, *Vol. 2. Bournemouth (UK): British Herbal Medicine Assoc.*
- **Briskin, D. (2000).** Medicinal Plants and Phytomedicines. Linking Plant Biochemistry and Physiology to Human Health. *Plant Physiol., 124: 507-514.*

Bubonja-Sonje, M., Giacometti, J., Abram, M., (2011). Antioxidant and antilisterial activity of olive oil, cocoa and rosemary extract polyphenols. *Food Chem. 127, 1821–1827.*

- **Buchner, N., Krumbein, A., Rohn, S., Kroh, L.W., (2006).** Effect of thermal processing on the flavonols rutin and quercetin. *Rapid Commun. Mass Spectrom. 20, 3229–3235.*
- **Buitimea-Cantúa, N.E., Gutiérrez-Uribe, J.A., Serna-Saldívar, S.O., (2017).** Phenolic–protein interactions: effects on food properties and health benefits. *J. Med. Food. https://doi.org/10.1089/jmf.2017.0057.*
- **Calabrese, V.; Scapagnini, G.; Catalano, C.; Dinotta, F.; Geraci, D. and Morganti, P. (2002).** Biochemical studies of a natural antioxidant isolated from rosemary and its application in cosmetic dermatology. *Int. J. of Tissue Reactions, 22 (1): 5-13.*
- **Cassandro, M., Comin, A., Ojala, M., Dal Zotto, R., De Marchi, M., Gallo, L., Carnier, P. & Bittante, G. (2008).** Genetic parameters of milk coagulation properties and their

relationships with milk yield and quality traits in Italian Holstein cows. *Journal of Dairy Science* 91(1), 371-376.

- **Charlton, A.J., Baxter, N.J., Khan, M.L., Moir, A.J.G., Haslam, E., Davies, A.P., Williamson, M.P., (2002).** Polyphenol/peptide binding and precipitation. *J. Agric. Food Chem. 50, 1593–1601*.
- **Chevallier, A. (1996).** The Encyclopedia of Medicinal Plants, *Dorling Kindersley, London.*
- **Chiej, R. (1984).** Encyclopedia of Medicinal Plants. *MacDonald ISBN 0-356-10541-5.*
- **Chiofalo, V., Maldonato, R., Martin, B., Dupont, D. & Coulon, J.B. (2000).** Chemical composition and coagulation properties of Modicana and Holstein cows' milk. *Annales de Zootechnie 49(6), 497-503.*
- **Christian, M.P., Grainger, C., Kefford, B. & Pearce, R.J. (1995).** Effect of cow diet and stage of lactation on casein and mineral composition of milk for cheese manufacture. *Journal of Dairy Science 78(Suppl. 1), 1) 133.*
- **Claesson, O. (1965).** Studies on the variation of the rennin coagulation time of milk. *Lantbrukshögskolans annaler 31, 237-332.*
- **Comin, A., Cassandro, M., Chessa, S., Ojala, M., Dal Zotto, R., De Marchi, M., Carnier, P., Gallo, L., Pagnacco, G. & Bittante, G. (2008).** Effects of composite β- and κ−casein genotypes on milk coagulation, quality, and yield traits in Italian Holstein cows. *Journal of Dairy Science 91(10), 4022-4027.*
- **Crozier, A., Jaganath, I.B., Clifford, M.N., (2009).** Dietary phenolics: chemistry, bioavailability and effects on health. *Nat. Product. Rep. 26, 1001–1043.*
- **Cui, L., Kim, M.O., Seo, J.H., Kim, I.S., Kim, N.Y., Lee, S.H., Park, J., Kim, J., Lee, H.S., (2012).** Abietane diterpenoids of Rosmarinus officinalis and their diacylglycerol acyltransferase-inhibitory activity. *Food Chem. 132, 1775–1780.*

- **Cuvelier, M. E.; Richard, H.; Berset, C. (1996).** Antioxidant Activity Phenolic Composition of Pilot-plant and Commercial Extracts of Sage and Rosemary. *J. Am. Oil Chem. Soc., 73: 645-652.*

- **Czubinski, J., Dwiecki, K., (2017).** A review of methods used for investigation of protein–phenolic compound interactions. *Int. J. Food Sci. Technol. 52, 573–585.*

- **da Conceicao Neta, E. R., Johanningsmeier, S., Drake, M., & McFeeters, R. (2007).** A chemical basis for sour taste perception of acid solutions and fresh-pack dill pickles. *Journal of food science, 72*(6), S352-S359. DOI: 10.1111/j.1750-3841.2007.00400.x.

- **Dannenberg, F. & Kessler, H.G. (1988a).** Effect of denaturation of β-lactoglobulin on texture properties of set-style nonfat yoghurt. II. Firmness and flow properties. *Milchwissenschaft 43(11), 700-704.*

- **de Raadt, P., Wirtz, S., Vos, E., & Verhagen, H. (2015).** Short review of extracts of rosemary as a food additive. *European Journal of Nutrition & Food Safety*, 126-137.

- **Del Bano, M. J.; Lorente, J.; Castillo, J.; Benavente-Garcia, O.; Marin, M. P.; Del Rio, J. A.; Ortuno, A. and Ibarra, I. (2004).** Flavonoid distribution during the development of leaves, flowers, stems, and roots of *Rosmarinus officinalis*. Postulation of a biosynthetic pathway. *J.Agric. Food Chem., 52(16): 4987-4992.*

- **Díaz-Maroto, M. C., P_erez eCoello, M. S., S_anchez- Palomo, E., & Gonz_alez Vi~nas, M. A. (2007).** Impact of drying and storage time on sensory characteristics of rosemary (Rosmarinus officinalis L.). *Journal of Sensory Studies, 22,34e48.*

- **DII: Dairy Industries International (2019).** Global cheese markets hit record highs. **Farkye, N. Y. (2004).** Cheese technology. *Internatinal Journal of Dairy Technology, 57 (2-3), 91-98.* dilution methods to determine the antibacterial activity of plant extracts. *J. Microbiol. Methods 81, 121–126.*

- **Djaoud, K., Boulekbache-Makhlouf, L., Yahia, M., Mansouri, H., Mansouri, N., Madani, K., & Romero, A. (2020).** Dairy dessert processing: Effect of sugar substitution by

date syrup and powder on its quality characteristics. *Journal of Food Processing and Preservation, 44*(5), e14414. doi: 10.1111/jfpp.14414

- **Djeridane A., Yousfi M., Nadjemi B., Boutassouna D., Stocker P. and Vidal N. (2006)**. Antioxidant activity of same Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, 97, pp: 654-660.
- **Dubeau, S., Samson, G., Tajmir-Riahi, H.A., (2010).** Dual effect of milk on the antioxidant capacity of green, Darjeeling and English breakfast teas. *Food Chem. 122, 539–545.*
- **Duke, J. A. (1997).** The Green Pharmacy. *Rodale Press. p. 507.*
- **Dumbrav_a, D.-G., Moldovan, C., Raba, D.-N., & Popa, M.-V. (2012).** Vitamin C, chlorophylls, carotenoids and xanthophylls content in some basil (Ocimum basilicum L.) and rosemary (Rosmarinus officinalis L.) leaves extracts. *Journal of Agroalimentary Processes and Technologies, 18(3), 253e258.*
- **Edwin N. Frankel, Shu-Wen Huang, Robert Aeschbachand Elizabeth Prior. (1996).** Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil-in-water emulsion. *Journal of Agricultural and Food Chemistry. 44(1):131-135.*
- **Elamrani, A., Zrira, S., Benjilali, B., & Berrada, M. (2000).** A study of Moroccan rosemary oils. *Journal of Essential Oil Research, 12, 487e495.*
- **ESCOP, (2003).** ESCOP Monographs: The Scientific Foundation for Herbal Medicinal Products, 2nded. Exeter (UK): *European Scientific Cooperative on Phytotherapy and Thieme.*
- **Exarchou, V.; Nenadis, N.; Tsimidou, M.; Gerothanassis, I. P.; Troganis, A. and Boskou, D. (2000).** Antioxidant activities and phenolic composition of extracts from Greek oregano, Greek sage, and summer savory. *J Agric. Food Chem., 50: 5294-5299.*

- **Felfoul, I., Borchani, M., Samet-Bali, O., Attia, H., & Ayadi, M. (2017).** Effect of ginger (Zingiber officinalis) addition on fermented bovine milk: Rheological properties, sensory attributes and antioxidant potential. *Journal of New Sciences, 44*(3), 2400-2409.

- **Felton, G.W., Broadway, R.M., Duffey, S.S., (1989).** Inactivation of protease inhibitor activity by plant-derived quinones: complications for host-plant resistance against noctuid herbivores. *J. Insect Physiol. 35, 981–990.*

- **Fernandes, P. A., Ferreira, S. S., Bastos, R., Ferreira, I., Cruz, M. T., Pinto, A., Coelho, E., Passos, C. P., Coimbra, M. A., & Cardoso, S. M. (2019).** Apple pomace extract as a sustainable food ingredient. *Antioxidants, 8*(6), 189.

- **Flüeler, O. (1978).** Research on slow-renneting milk. I. Composition of the milk. *Schweizerische Milchwirtschaftliche Forschung* 7(3), 45-54.

- **Fox, P. F. & McSweeney, P. L. H. (2004).** Cheese: an overview. In P. F. Fox, P. L. H. McSweeney, T. M. Cogan & T. P. Guinee, *Cheese: Chemistry, Physics and Microbiology, Vol. 1 (pp. 1-18). UK: Academic Press.*

- **Frazier, R.A., Deaville, E.R., Green, R.J., Stringano, E., Willoughby, I., Plant, J., Mueller-Harvey, I., (2010).** Interactions of tea tannins and condensed tannins with proteins. *J. Pharm. Biomed. Anal. 51, 490–495.*

- **Frazier, R.A., Papadopoulou, A., Green, R.J., (2006).** Isothermal titration calorimetry study of epicatechin binding to serum albumin. *J. Pharm. Biomed. Anal. 41, 1602–1605.*

- **Frutos MJ, Hernández-Herrero JA.** (**2005).** Effects of rosemary extract (*Rosmarinus officinalis*) on the stability of bread with an oil, garlic and parsley dressing. *LWT-Food Science and Technology 38(6):651-655.* https://doi.org/10.1016/j.lwt.2004.08.013

- **Gad, A. S., & El-Salam, M. H. A. (2010).** The antioxidant properties of skim milk supplemented with rosemary and green tea extracts in response to pasteurisation, homogenisation and the addition of salts. *International Journal of Dairy Technology, 63*(3), 349-355.

- **Gad, A. S., & Sayd, A. F. (2015).** Antioxidant properties of rosemary and its potential uses as natural antioxidant in dairy products—A review. *Food and Nutrition Sciences, 6(01), 179.*

- **Gad, A.S., Ghita, I.E., El-Din, H.M.F., Badran, S.M.A. and El-Messery, T.M. (2010)**. Production Health Benefits Yoghurt Supplemented with Rosemary and Green Tea Extract as Natural antioxidant. *IDF World Dairy Summit, Auckland, 5-11.*

- **Georgé, S., Brat, P., Alter, P., & Amiot, M. J. (2005).** Rapid determination of polyphenols and vitamin C in plant-derived products. *J Agric Food Chem*, *53*(5), 1370-1373.

- **Goodwin, T. W. and Mercer, E.I. (1983).** Introduction to Plant Biochemistry. Pergamon, New York.

- **Gornal Allan G., Bardawi Charles J. and David Maxima M. (1949).** Determination of serum proteins by means of the biuret reaction.

- **Granato, D., Nunes, D. S., & Barba, F. J. (2017).** An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statistics in the development of functional foods: A proposal. *Trends in Food Science & Technology*, *62*, 13-22.

- **Granato, D., Nunes, D. S., & Barba, F. J. (2017).** An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statistics in the development of functional foods: A proposal. *Trends in Food Science & Technology*, *62*, 13-22.

- **Grandison, A.S. & Ford, G.D. (1986).** Effects of variations in somatic cell count on the rennet coagulation properties of milk and on the yield, composition and quality of Cheddar cheese. *Journal of Dairy Research* 53(4), 645-655.

- **Gregory JR, DL Collins, PSW Davies & JM Hughes.** (**1995).** National diet and nutrition survey: Children aged. 1.5 to 4.5 years, report of the diet and nutrition survey. *London: HMSO.*

- **Guo, J., Yue, T., & Yuan, Y. (2019).** Impact of polyphenols on the headspace concentration of aroma compounds in apple cider. *Journal of the Science of Food and Agriculture, 99*(4), 1635-1642. https://doi.org/10.1002/jsfa.9345.

- **Guo, W., Kong, E., Meydani, M., (2009).** Dietary polyphenols, inflammation, and cancer. *Nutr. Cancer 61, 807–810.*

- **Guyomarc'h, F., Law, A.J.R. & Dalgleish, D.G. (2003a).** Formation of soluble and micelle bound protein aggregates in heated milk. *Journal of Agricultural and Food Chemistry* 51(16), 4652-4660.

- **Hagerman A. E. and Butler L. G. (1978)**. Protein precipitation method for quantitative determination of tannins. *Journal of Agriculture and Food Chemistry, 26, pp: 809-812.*

- **Hagerman, A.E., (1989).** Chemistry of tannin-protein complexation. In: **Hemingway, R.W., Karchesy, J.J. (Eds.)**. Chemistry and Significance of Condensed Tannins. *Plenum Press, New York, p. 323.*

- **Hala, M., Ebtisam, E., Sanaa, I., Badran, M., Marwa, A., & Said, M. (2010).** Manufacture of low fat UF-soft cheese supplemented with rosemary extract (as natural antioxidant). *J. Am. Sci., 6*, 570-579.

- **Haloui, M.; Louedec, L.; Michel, J. B. and Lyoussi, B. (2000).** Experimental diuretic effects of *Rosmarinus officinalis* and *Centaurium erythraea*. J. Ethnopharmacol., 71: 465-472.

- **Haque, Z. & Kinsella, J.E. (1988).** Interaction between heated κ-casein and β-lactoglobulin: predominance of hydrophobic interactions in the initial stages of complex formation. *Journal of Dairy Research* 55(1), 67-80.

- **Harborne, J. B. (1973).** Phytochemical Methods. *Science paper backs, Chapman Hall, London.*

- **Hartmann, T. (1996).** Diversity and variability of plant secondary metabolism: a mechanistic view. *Entomol. Exp. Appl., 80: 177-188.*

- **Hedge, I. C. (1992).** A global survey of the biogeography of the Labiatae. In: Harley, R.M., 666 Reynolds, T. (eds.) *Advances in Labiate science. Kew: Royal Botanic Gardens, pp. 7–17.*

- **Heinrich, M., Kufer, J., Leonti, M., & Pardo-de-Santayana, M. (2006).** Ethnobotany and ethnopharmacology and Interdisciplinary links with the historical sciences. *Journal of Ethnopharmacology, 107, 157e160.*

- **Henderson L., J Gregory, G Swan.** (**2002).** The national diet & nutrition survey: Adults aged 19 to 64 years. *HM Stationery Office.*

- **Hernández-Hernández E., E. Ponce-Alquicira, M.E.Jaramillo-Flores, I. Guerrero Legarreta. (2009).** Antioxidant effect rosemary (*Rosmarinus officinalis* L.) and oregano (*Origanum vulgare* L.) extracts on TBARS and colour of model raw pork batters. *Meat Science. 81(2):410-417.*

- **Ho, C. T.; Ferraro, T.; Chen, Q.; Rosen, R. T. and Huang, M. T. (1994).** Phytochemicals in teas, rosemary, and their cancer preventive properties. In **Ho, C.-T.; Osawa, T.; Huang, M.-T. and Rosen, R. T. (eds).** Food Phytochemicals for Cancer Prevention 2. Am. Chem. Soc. Sym. Series, 547. *American Chemical Society, Washington, DC, 2-19.*

- **Hoefler, C.; Fleurentin, J.; Mortier, F.; Pelt, J. M. and Guillemain, J. J. (1987).** Comparative choleretic and hepatoprotective properties of young sprouts and total plant extracts of *Rosmarinus officinalis* in rats. J. Ethnopharmacol., 19: 133-143.

- **Hosseinimehr, S.J., Ahmadi, A., Beiki, D., Habibi, E., Mahmoudzadeh, A., (2009).** Protective effects of hesperidin against genotoxicity induced by 99mTc-MIBI in human cultured lymphocyte cells. *Nucl. Med. Biol. 36, 863–867.*

- **Hwang, C. & Gunasekaran, S. (2001).** Measuring crumbliness of some commercial Queso Fresco-type Latin American cheeses. *Milchwissenschaft, 56(8), 446-450.*

- **Ikonen, T., Ahlfors, K., Kempe, R., Ojala, M. & Ruottinen, O. (1999a).** Genetic parameters for the milk coagulation properties and prevalence of non-coagulating milk in Finnish dairy cows. *Journal of Dairy Science* 82(1), 205-214.
- **Ikonen, T., Morri, S., Tyrisevä, A.M., Ruottinen, O. & Ojala, M. (2004).** Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content, and pH of milk. *Journal of Dairy Science* 87(2), 458-467.
- **Irina I. Koleva, Teris A. van Beek , Jozef P. H. Linssen , Aede de Groot Lyuba N. Evstatieva. (2002).** Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochemical Analysis. 13(1):8-17.*
- **Ivanović Jasna, Sonja Đilas, Milka Jadranin, Vlatka Vajs, Nada Babović, Slobodan Petrović and Irena Žižović (2009).** Supercritical carbon dioxide extraction of antioxidants from rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.). *J. Serb. Chem. Soc. 74* (7) 717–732. *JSCS–3870.*
- **Jakobek, L., (2015).** Interactions of polyphenols with carbohydrates, lipids and proteins. *Food Chem. 175, 556–567.*
- **Jongberg, S., Lund, M.N., Otte, J., (2015).** Dissociation and reduction of covalent b-lactoglobulin-quinone adducts by dithiothreitol, tris(2-carboxyethyl)phosphine, or sodium sulfite. *Anal. Biochem. 478, 40–48.*
- **Jõudu, I., Henno, M., Varv, S., Kaart, T., Kart, O. & Kalamees, K. (2007).** Milk protein genotypes and milk coagulation properties of Estonian native cattle. *Agricultural and Food Science* 16, 222-231.
- **Kaloustian, J., Portugal H., Pauli A. M., Pastor J. (2001).** Chemical, Chromatographic, and Thermal Analysis of Rosemary (Rosmarinus officinalis). *Journal of Applied Polymer Science, Vol. 83, 747–756.*
- **Karabegović, I. T., Stojičević, S. S., Veličković, D. T., Nikolić, N. Č., & Lazić, M. L. (2013).** Optimization of microwave-assisted extraction and characterization of phenolic

compounds in cherry laurel (Prunus laurocerasus) leaves. *Separation and Purification Technology, 120,* 429-436.

- **Karabegović, I. T., Stojičević, S. S., Veličković, D. T., Todorović, Z. B., Nikolić, N. Č., & Lazić, M. L. (2014).** The effect of different extraction techniques on the composition and antioxidant activity of cherry laurel (Prunus laurocerasus) leaf and fruit extracts. *Industrial Crops and Products, 54,* 142-148.
- **Kikuko, T.; Shigemi, S.; Masahiro, S. and Tatsu, M. (1993).** Hair-raising cosmetic. *Japanese Patent no. 05286835*.
- **Klanˇcnik, A., Piskernik, S., Jerˇsek, B., Moˇzina, S.S., (2010).** Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. *Journal of Microbiological Methods 81:121-126.*
- **Koestler, A.G. (1925).** Different types of milk, their relation to rennet, and their importance in cheese making. *Journal of Dairy Science 8(1), 28-36.*
- **Kontogianni, V.G., Tomic, G., Nikolic, I., Nerantzaki, A., Sayyad, A., Stosic-Grujicic, N., Stojanovic, S., Gerothanassis, I., Tzakos, I.PA.G. (2013).** Phytochemical profile of *Rosmarinus officinalis* and *Salvia officinalis* extracts and correlation to their antioxidant and anti-proliferative activity. *Food Chem. 136 (1), 120–129.*
- **Kosaka, K. and Yokoi, T. (2003).** Carnosic acid, a component of rosemary (*Rosmarinus officinalis* L.), promotes synthesis of nerve growth factor in T98G human glioblastoma cells. Biol. Pharmcol. Bull., 26: 1620-1622.
- **Kovar, K. A.; Gropper, B.; Friess, D. and Ammon, H. P. T. (1987).** Blood levels of 1, 8-cineoile and locomotor activity of mice after inhalation and oral administration of rosemary oil. Planta Med., 53: 315-318.

- **Kroll, J., Rawel, H.M., Rohn, S., (2003).** Reactions of plant phenolics with food proteins and enzymes under special consideration of covalent bonds. *Food Sci. Technol. Res. 9, 205–218.*

- **L. A. Jimenez-Maroto, A. Lopez-Hernandez, D. L. Borneman, and S. A. Rankin (2016).** A comparison of fresh, *pasta filata*, and aged Hispanic cheeses using sensory, chemical, functional, and microbiological assessments. *Journal of Dairy Science Vol. 99 No. 4.* http://dx.doi.org/10.3168/jds.2015-10112.

- **Larsen, L.B., Rasmussen, M.D., Bjerring, M. & Nielsen, J.H. (2004).** Proteases and protein degradation in milk from cows infected with Streptococcus uberis. *International Dairy Journal 14(10), 899-907.*

- **Le Bourvellec, C., Renard, C.M.G.C., (2012).**Interactions between polyphenols and macromolecules: quantification methods and mechanisms. Crit. Rev. *Food Sci. Nutr. 52, 213–248.*

- **Leitner, G., Krifucks, O., Merin, U., Lavi, Y. & Silanikove, N. (2006).** Interactions between bacteria type, proteolysis of casein and physico-chemical properties of bovine milk. *International Dairy Journal 16(6), 648-654.*

- **Lemos, M. F., Lemos, M. F., Pacheco, H. P., Endringer, D. C., & Scherer, R. (2015).** Seasonality modifies rosemary's composition and biological activity. *Industrial Crops and Products, 70, 41-47.*

- **Leung, A. Y. and Foster, S. (1996).** Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics. *2nded. New York: Wiley-Interscience, 446-448.*

- **Linares, I. B., Arráez-Román, D., Herrero, M., Ibáñez, E., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2011).** Comparison of different extraction procedures for the comprehensive characterization of bioactive phenolic compounds in Rosmarinus officinalis by reversed-phase high-performance liquid chromatography with diode array detection coupled to

electrospray time-of-flight mass spectrometry. *Journal of Chromatography A, 1218(42), 7682-7690.*

- **Lindström, U.B., Antila, V. & Syväjärvi, J. (1984).** A note on some genetic and non-genetic factors affecting clotting time of Ayrshire milk. *Acta Acriculturae Scandinavica 34, 349-355.*

- **Loranty, A., Rembiałkowska, E., Rosa, E. S., & Bennett, R. N. (2010).** Identification, quantification and availability of carotenoids and chlorophylls in fruit, herb and medicinal teas. *Journal of Food Composition and Analysis, 23, 432e441.*

- **Lorenzi, H., & Matos, F. J. (2006).** Plantas medicinais no Brasil: Nativas e ex_oticas cultivadas. *Primeira Ediç~ao. Instituto Plantarum. Nova Odessa, 512 pp.*

- **Losi, G., Castagnetti, G.B., Morini, D., Resmini, P., Volonterio, G. & Mariani, P. (1982).** Milk with abnormal coagulation: chemical and physico-chemical factors. *Industria Del Latte 18(1), 13-33.*

- **Loussouarn, M., Krieger-Liszkay, A., Svilar, L., Bily, A., Birtić, S., & Havaux, M. (2017).** Carnosic acid and carnosol, two major antioxidants of rosemary, act through different mechanisms. *Plant physiology, 175*(3), 1381-1394.

- **Lucey, J.A. & Singh, H. (1997).** Formation and physical properties of acid milk gels: A review. *Food Research International* 30(7), 529-542.

- **Luis Almela, Blas Sanchez-Munoz, Jose A. Fern andez-Lopez, Maria J. Roca, Virginia Rabe (2006).** Liquid chromatograpic–mass spectrometric analysis of phenolics and free radical scavenging activity of rosemary extract from different raw material. *Journal of Chromatography A. 1120(1–2):221-229.*

- **Lukaczer, D.; Darland, G.; Tripp, M.; Liska, D.; Lerman, R. H.; Schiltz, B. and Bland, J. S. (2005).** A pilot trial evaluating Meta050, a proprietary combination of reduced iso-alpha acids, rosemary extract and oleanolic acid in patients with arthritis and fibromyalgia. *Phytother. Res., 19: 864-869.*

- **Lund, C. M., Nicolau, L., Gardner, R. C., & Kilmartin, P. A. (2009).** Effect of polyphenols on the perception of key aroma compounds from Sauvignon Blanc wine. *Australian Journal of Grape and Wine Research, 15*(1), 18-26. doi: 10.1111/j.1755-0238.2008.00028.x

- **Luqman, S.; Dwivedi, G. R.; Darokar, M. P.; Kalra, A. and Khauuja, S.P.S. (2007).** Potential of rosemary oil to be used in drug-resistant infections. *J. Alternative Therapies in Health and Medicine, 13(5): 54-59.*

- **Luquet F, (1990).** Laits et produits laitiers vache brebis chèvre : les produits laitiers transformation et technologies. *Ed 2 tec & doc-Lavoisier.100p.*

- **Luquet François-Marie, Corrieu Georges. (2005).** Bactéries lactiques et probiotiques, Collection Science & Technique Agro-alimentaire, Editions Tec & Doc. PP 3-7.

- **Ly, T. N.; Shimoyamada, M. and Yamauchi, R. (2006).** Isolation and characterization of rosmarinic acid oligomers in *Celastrus hindsii* Benth leaves and their antioxidative activity. *Food. Chem., 54: 3786-3793.*

- **M. S. Santana, F. F. Bezerril, M. S. Madruga, A. S. M. Batista, M. Magnani, E. L. Souza, and R. C. R. E. Queiroga (2013).** Nutritional and sensory characteristics of Mina's fresh cheese made with goat milk, cow milk, or a mixture of both. *J. Dairy Sci. 96:7442–7453* http://dx.doi.org/ 10.3168/jds.2013-6915.

- **Macheboeuf, D., Coulon, J.B. & D'Hour, P. (1993).** Effect of breed, protein genetic variants and feeding on cows' mik coagultating properties. *Journal of Dairy Research* 60, 43-54.

- **Madina Naimi, Filip Vlavcheski, Hesham Shamshoum and Evangelia Tsiani (2017).** Rosemary Extract as a Potential Anti-Hyperglycemic Agent: Current Evidence and Future Perspectives. *Review, Nutrients 9, 968; doi: 10.3390/nu9090968.*

- **Mahaut M, Jeantet R, Brule G, Schuck P, (2000).** Les produits industriels laitiers. Pp .180.

- **Malencic, D. J.; Gasic, O.; Popovic, M. and Boza, P. (2000).** Screening for antioxidant properties of *Salvia reflexa* Hornem. Phytother. Res., 14: 546-548.
- **Malossini, F., Bovolenta, S., Piras, C., DallaRosa, M. & Ventura, W. (1996).** Effect of diet and breed on milk composition and rennet coagulation properties. *Annales de Zootechnie 45(1), 29-40.*
- **Mann, J. (1987).** Secondary Metabolism. *Oxford University Press. U.K.*
- **Maran, J. P., Sivakumar, V., Thirugnanasambandham, K., & Sridhar, R. (2013).** Optimization of microwave assisted extraction of pectin from orange peel. *Carbohydrate polymers, 97*(2), 703-709.
- **Maran, J. P., Sivakumar, V., Thirugnanasambandham, K., & Sridhar, R. (2014).** Microwave assisted extraction of pectin from waste Citrullus lanatus fruit rinds. *Carbohydrate polymers, 101*, 786-791.
- **Marcelo D. Catarino, Artur M.S. Silva, Susana C. Saraiva, Abilio J.F.N. Sobral, Susana M. Cardoso (2015).** Characterization of phenolic constituents and evaluation of antioxidant properties of leaves and stems of Eriocephalus africanus. *Arabian Journal of Chemistry. King Saud University.*
- **Maria Cristina Delgado Da Silva, Alecia Cristinne Santos Ramos, Izildinha Moreno, Juliana De Oliveira Moraes (2010).** Influence of the production procedures on the physic-chemical, sensory and microbiological characteristics of *coalho* cheese. *Rev Inst Adolfo Lutz. 69(2214-21.*
- **Marin, M., Koko, V., Duleti_c-Lau_sevi_c, A., Marin, P. D., Ran_ci_c, D., & Dajic-Stevanovic, Z. (2006).** Glandular trichomes on the leaves of *Rosmarinus officinalis*: morphology,stereology and histochemistry. *South African Journal of Botany, 72, 378e382*.
- **Martini, S., Claudia, B., Claudio, R., (2008).** Interaction of quercetin and its conjugate quercetin 3-O-b-D-glucopyranoside with albumin as determined by NMR relaxation data. *J. Nat. Prod. 71, 175–178.*

- **Masuda, T.; Inaba, Y.; Maekawa, T.; Takeda, Y.; Tamura, H. and Yamaguchi, H. (2002).** Recovery mechanism of the antioxidant activity from carnosic acid quinone, and oxidized sage and rosemary antioxidant. *J. Agric. Food Chem., 50: 5863-5869.*

- **McKenzie, G.H., Norton, R.S., Sawyer, W.H., Marziali, A.S. & Ng-Kwai-Hang, K.F. (1971).** Heat-induced interaction of β-lactoglobulin and κ-casein. *Journal of Dairy Research* 38(3), 343-351.

- **McRae, J.M., Kennedy, J.A., (2011).** Wine and grape tannin interactions with salivary proteins and their impact on astringency: a review of current research. *Molecules 16, 2348–2364.*

- **Milan Stankovic (2020).** Lamiaceae Species: Biology, Ecology and Practical Uses. DOI: 10.3390/books978-3-03928-419-1 Edition: Printed Edition of the Special Issue Published in *Plants Publisher: MDPIISBN: ISBN 978-3-03928-418-4 (Pbk); ISBN 978-3-03928-419-1*

- **Mills, S. and Bone, K. (2005).** The Essential Guide to Herbal Safety. *St. Louis (MO): Elsevier Churchill Living stoes.*

- **Miraldi, E., Giachetti, D., Mazzoni, G., & Biagi, M. (2010).** Quali-quantitative analysis of eight Rosmarinus officinalis essential oils of different origin. First report. *Journal of the Siena Academy of Sciences, 42-43.*

- **Moreno, S., Ojeda Sana, A. M., Gaya, M., Barni, M. V., Castro, O. A., & Van Baren, C. (2012).** Rosemary compounds as nutraceutical health products. In Y. El-Samragy (Ed.), Food additives chapter 9. Rijeka, Croatia: *Intech-open Science.*

- **Moreno, S.; Scheyer, T.; Romano, C. S. and Vojnov, A. A. (2006).** Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radical Res., 40 (2): 223-231.

- **Mulaudzi, R.B., Ndhlala, A.R., Kulkarni, M.G., Staden, J.V., (2012).** Pharmacological properties and protein binding capacity of phenolic extracts of some Venda medicinal plants used against cough and fever. *J. Ethnopharmacol. 143, 185–193.*

- **Muniandy, P., Shori, A. B., & Baba, A. S. (2016).** Influence of green, white and black tea addition on the antioxidant activity of probiotic yogurt during refrigerated storage. *Food Packaging and Shelf Life, 8, 1-8.*

- **Munne-Bosch, S. and Alegre, L. (2001).** Subcellular Compartmentation of the Diterpene Carnosic Acid and Its Derivatives in the Leaves of Rosemary. American Society of Plant Physiologists, *Plant Physiol., 125 (2): 1094-1102.*

- **Naczk, M., Grant, S., Zadernowski, R., Barre, E., (2006).** Protein precipitating capacity of phenolics of wild blueberry leaves and fruits. *Food Chem. 96, 640–647.*

- **Naczk, M., Oickle, D., Pink, D., Shahidi, F., (1996).** Protein precipitating capacity of crude canola tannins: effect of pH, tannin, and protein concentrations. *J. Agric. Food Chem. 44, 2144–2148.*

- **Nadkarni, A. (1994).** Dr. K.M. Nadkarini's India Materia Medica. 3rded. Popular Prakashan Bombay, India. Stefanovits-Banyai, E.; Tulok, M. H.; Hegedus, A.; Renne, C. and Varga, I. S. (2003). Antioxidant effect of various rosemary (*Rosmarinus officinalis*) clones. *Acta. Biol. Szeged., 47(1-4):111-113.*

- **Newall, C.; Anderson, L. and Phillipson, J. (1996).** Herbal Medicines: A Guide for Health Care Professionals. *London: Pharmaceutical Press, 229-230.*

- **Okamura, N.; Fujimoto, Y.; Kuwabara, S. and Yagi, A. (1994a).** High performance liquid chromatographic determination of carnosic acid and carnosol in *Rosmarinus offficinalis* and *Salvia officinalis*. *J.Chromatogr., 679: 381-386.*

- **Okazaki, T., Suetsugu, M., and Yoshida, T. (1989).** Hair tonics containing oleanolic derivatives. *Chemical Abstracts, p: 107.*

- **Okigbo, L.M., Richardson, G.H., Brown, R.J. & Ernstrom, C.A. (1985c).** Variation in coagulation properties of milk from individual cows. *Journal of Dairy Science 68(4), 822-828.*

- **Okigbo, L.M., Richardson, G.H., Brown, R.J. & Ernstrom, C.A. (1985a).** Coagulation properties of abnormal and normal milk from individual cow quarters. *Journal of Dairy Science 68(8), 1893-1896.*

- **Okigbo, L.M., Richardson, G.H., Brown, R.J. & Ernstrom, C.A. (1985b).** Effects of pH, calcium chloride and chymosin concentration on coagulation properties of abnormal and normal milk. *Journal of Dairy Science 68(10), 2527-2533.*

- **Oluwatuyi, M.; Kaatz, G. W. and Gibbons, S. (2004).** Antibacterial and resistance modifying activity of *Rosmarinus officinalis*. Phytochem, *65:3249-3254.*

- **Osakabe, N.; Yasuda, A.; Natsume, M. and Yoshikawa, T. (2004).** Rosmarinic acid inhibits epidermal inflammatory responses: anticarcinogenic effect of *Perilla frutescens* extract in the murine two-stage skin model. *Carcinogenesis. 25: 549-557.*

- **Ostersen, S., Foldager, J. & Hermansen, J.E. (1997).** Effects of stage of lactation, milk protein genotype and body condition at calving on protein composition and renneting properties of bovine milk. *Journal of Dairy Research 64(2), 207-219.*

- **Ozcan, M. M., Ünver, A., Uçar, T., & Arslan, D. (2008).** Mineral content of some herbs and herbal teas by infusion and decoction. *Food Chemistry, 106(3), 1120e1127.*

- **Ozdal, T., Capanoglu, E., Altay, F., 2013.** A review on protein–phenolic interactions and associated changes. *Food Res. Int. 51, 954–970.*

- **Ozdal, T., Capanoglu, E., Altay, F., 2013.** A review on protein–phenolic interactions and associated changes. *Food Res. Int. 51, 954–970.*

- **Pan, X., Niu, G., & Liu, H. (2003).** Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chemical Engineering and Processing: Process Intensification, 42(2), 129-133.*

- **Papadopoulou, A., Green, R.J., Frazier, R.A., (2005).** Interaction of flavonoids with bovine serum albumin: a fluorescence quenching study. *J. Agric. Food Chem. 53, 158–163.*

- **Parada, J., Aguilera, J.M., (2007).** Food microstructure affects the bioavailability of several nutrients. *J. Food Sci. 72, 21–32.*

- **Paris, A.; Strukelj, B.; Renko, M., Turk, V., Pukl, M., Umek, A. and Korant, B. D. (1993).** Inhibitory effect of carnosic acid on HIV-1 protease in cell-free assays. *J. Nat. Prod., 56(8):1426-1430.*

- **Pathare, P. B., Opara, U. L., & Al-Said, F. A.-J. (2013).** Colour measurement and analysis in fresh and processed foods: a review. *Food and Bioprocess Technology, 6*(1), *36-60.*

- **Patras, A., Brunton, N. P., Tiwari, B., & Butler, F. (2011).** Stability and degradation kinetics of bioactive compounds and colour in strawberry jam during storage. *Food and Bioprocess Technology, 4*(7), 1245-1252.

- **Paul de Raadt1, Sabrina Wirtz1, Ellen Vos1 and Hans Verhagen (2015).** Short Review of Extracts of Rosemary as a Food Additive. *European Journal of Nutrition & Food Safety 5(3): 126-137 Article no.EJNFS.012 ISSN: 2347-5641*

- **Pintore, G., Usai, M., Bradesi, P., Juliano, C., Boatto, G., Tomi, F., et al. (2002).** Chemical composition and antimicrobial activity of Rosmarinus officinalis L. oils from Sardinia and Corsica. *Flavour and Fragrance Journal, 17, 15e19.*

- **Piskernik, S., Klanˇcnik, A., Riedel, C.T., Brøndsted, L., Mozina, S.S., (2011).** Reduction of Campylobacter jejuni by natural antimicrobials in chicken meat-related conditions. *Food Control 22, 718–724.*

- **Poncet-Legrand, C., Edelmann, A., Putaux, J.L., Cartalade, D., Sarni-Manchado, P., Vernhet, A., (2006).** Poly (L-proline) interactions with flavan-3-ols units: influence of the molecular structure and the polyphenol/protein ratio. *Food Hydrocoll. 20, 687–697.*

- **Prigent, S., (2005).** Interactions of Phenolic Compounds with Globular Proteins and Their Effects on Food-related Functional Properties. *Wageningen University (Ph.D. thesis).*

- **Prigent, S.V.E., Gruppen, H., Visser, A.J.W.G., Van Koningsveld, G.A., Alfons, G.J.V., (2003).** Effects of non-covalent interactions with 5-O-caffeoylquinic acid (CGA) on the heat denaturation and solubility of globular proteins. *J. Agric. Food Chem. 51, 5088–5095.*

- **Ramdane Sidali, Brahim Mahmoud, Tlemsani Amine, Djermoun Abdelkader et Hadjsadok Tahar (2019).** Quelles disparités de consommation du lait et produits laitiers en algérie á travers les régions? *Revue Agrobiologia (2019) 9(1): 1449-1457, ISSN (Online): 2507-7627.*

- **Ramirez-Navas, J. S., & Rodriguez de Stouvenel, A. (2012).** Characterization of Colombian quesillo cheese by spectrocolorimetry. *Vitae, 19*(2), 178-185.

- **Rawel, H., Rohn, S., (2010).** Nature of hydroxycinnamate-protein interactions. *Phytochem. Rev. 9, 93–109.*

- **Rawel, H.A., Meidtner, K., Kroll, J., (2005).** Binding of selected phenolic compounds to proteins. *J. Agric. Food Chem. 53, 4228–4235.*

- **Ray B. (1996).** Probiotics of lactic acid bacteria: science or myth .In**:** Bozoglu TF et Ray B**.** (Eds.), *Springer-Verlag Berlin Heidelberg. Germany, p 101-136.*

- **Regiane Ribeiro-Santos, Denise Carvalho-Costa, Carlos Cavaleiro, Helena S. Costa, Tânia Gonçalves Albuquerque, Maria Conceiçao Castilho, Fernando Ramos, Nath_alia R. Melo and Ana Sanches-Silva (2015).** A novel insight on an ancient aromatic plant: The rosemary (Rosmarinus officinalis L.). *Trends in Food Science & Technology 45-355-368.*

- **Renata TiekoNassu[a]Lireny Aparecida GuaraldoGonçalves[b]Maria Aparecida AzevedoPereira da Silva[b]Frederico JoséBeserra. (2003).** Oxidative stability of fermented goat meat sausage with different levels of natural antioxidant. *Meat Science. 63(1):43-49.*

- **Richonnet Céline**. **(2015)** Caractéristiques nutritionnelles des fromages fondus. *Edition Elsevier Masson., p 9.*

- **Rita de Cássia Ramos do Egypto Queiroga, Bárbara Melo Santos, Ana Maria Pereira Gomes, Maria João Monteiro, Susana Maria Teixeira, Evandro Leite de Souza, Carlos José Dias Pereira, Maria Manuela Estevez Pintado (2013).** Nutritional, textural and sensory properties of Coalho cheese made of goats', cows' milk and their mixture. *LWT - Food Science and Technology 50 (2013) 538e544.*

- **Rossell_o, J. A., Cosín, R., Boscaiu, M., Vicente, O., Martıínez, I., & Soriano, P. (2006).** Intragenomic diversity and phylogenetic systematics of wild rosemaries (Rosmarinus officinalis L. s.l., Lamiaceae) assessed by nuclear ribosomal DNA sequences (ITS). *Plant Systematics and Evolution, 262, 1e12.*

- **Santana-Méridas O., Polissiou M., Izquierdo-Melero M.E., Astraka K., Petros A. Tarantilis, Herraiz-Penalver D., Sánchez-Vioque R. (2014).** Polyphenol composition, antioxidant and bioplaguicide activities ofthe solid residue from hydrodistillation of Rosmarinus officinalis L. *Industrial Crops and Products 59 :125–134.*

- **Santoyo, S.; Cavero, S.; Jaime, L.; Ibanez, E.; Senorans, F. J. and Reglero, G. (2005).** Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. essential oil obtained via supercritical fluid extraction. *J. Food Prot., 68(4): 790-795.*

- **Sastry, M.C.S., Rao, M.S.N., (1990).** Binding of CGA by the isolated polyphenol-free 11S protein of sunflower (Helianthus annus) seed. *J. Agric. Food Chem. 38, 2103–2110.*

- **Sawyer, W.H. (1969).** Complex between β−lactoglobulin and κ−casein. A review. *Journal of Dairy Science 52(9), 1347-1355.*

- **Schaar, J. (1984).** Effects of κ-casein genetic variants and lactation number on the renneting properties of individual milks. *Journal of Dairy Research 51(3), 397-406.*

- **Scherer, R., Godoy, H.T. (2009).** Antioxidant activity index (IAA) by 2,2-diphenyl-1-picrylhydrazyl method. *Food Chem. 112, 654–658.*

- **Schwarz, K. and Ternes, W. (1992).** Antioxidative constituents of *Rosmarinus officinalis* and *Salavia officinalis* I. Z. Lebensm. *Unters. Forsch. 195: 95-98.*

- **Sebranek J.G. V.J.H.Sewalt, K.L.Robbins, T.A.Houser. (2005).** Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. *Meat Science. 69(2):289- 296.*

- **Senge, B., Schulz, D. & Krenkel, K. (1997).** Structure formation in milk during rennet coagulation. *Applied Rheology* 7(4), 153-160.

- **Shan, B., Cai, Y. Z., Sun, M., & Corke, H. (2005).** Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *J Agric Food Chem, 53(20), 7749-7759.*

- **Shetty, K. (l997).** Biotechnology to harness the benefits of dietary phenolics; Focus on Lamiaceae. Asia Pac. *J. Clin. Nutr., 6: 162-171.*

- **Siebert, K.J., (1999).** Reviews-effect of protein-polyphenol interactions on beverage haze, stabilization and ana1ysis. *J. Agric. Food Chem. 47, 353–362.*

- **Siebert, K.J., (2006).** Hazes formation in beverages. *LWT Food Sci. Technol. 39, 987–994.*

- **Singletary, K. Singletary, K., MacDonald, C., Wallig, M. and Fisher, C. (1996).** Inhibition by rosemary and carnosol of 7, 12-dimethylbenz[a]anthracene (DMBA)-induced rat mammary tumorgenesis and *in vivo* DMBA-DNA adduct formation. *Normal. Cancer Lett., 103(2): 137-141.*

- **Slamenova, D., Kuboskova, K., Horvathova, E. and Robichova, S. (2002).** Rosemary-stimulated reduction of DNA strand breaks and FPGsensitive sites in mammalian cells treated with H2O2 or visible lightexcited methylene blue. *Cancer Lett., 177: 145-153.*

- **Small, E. (1997).** Culinary Herbs, National Research Council of Canada, NRC Press Ottawa.

- **Sotelo-Felix, J.I., Martinez-Fong, D. and Muriel De la Torre, P. (2002).** Protective effect of carnosol on CCl4-induced acute liver damage in rats. Eur. *J. Gastroenterol. Hepatol., 14:1001-1006.*

- **Sotelo-Felix, J.I., Martinez-Fong, D. and Muriel De la Torre, P. (2002).** Protective effect of carnosol on CCl4-induced acute liver damage in rats. Eur. *J. Gastroenterol. Hepatol., 14:1001-1006.*

- **Souza, L.C., de Gomes, M.G., Goes, A.T.R., Del Fabbro, L., Filho, C.B., Boeira, S.P., Jesse, C.R., (2013).** Evidence for the involvement of the serotonergic 5-HT1A receptors in the 2 antidepressant-like effect caused by hesperidin in mice. Prog. Neuro-Psychopharmacol. *Biol. Psychiatry 40, 103–109.*

- **Steven L. Richheimer, Matthew W. Bernart, Greg A. King, Michael C. Kent & David T. Beiley. (1996).** Antioxidant activity of lipid-soluble phenolic diterpenes from rosemary. *Journal of the American Oil Chemists' Society. 73(4):507-514.*

- **Strauss, G., Gibson, S.M., (2004).** Plant phenolics as cross-linkers of gelatin gels and gelatin based coacervates for use as food ingredients. *Food Hydrocoll. 18, 81–89.*

- **Švarc-Gajić, J., Stojanović, Z., Segura Carretero, A., Arráez Román, D., Borrás, I., & Vasiljević, I. (2013).** Development of a microwave-assisted extraction for the analysis of phenolic compounds from Rosmarinus officinalis. *Journal of Food Engineering, 119(3), 525-532*. doi: 10.1016/j.jfoodeng.2013.06.030.

- **Szumny, A., Figiel, A., Guti_errez-Ortíz, A., & Carbonell-Barrachina, _A. A. (2010).** Composition of rosemary essential oil (Rosmarinus officinalis) as affected by drying method. *Journal of Food Engineering, 97(2), 253e260.*

- **Taiz, L. and Zeiger, E. (2002).** Plant Physiology. Sinauer Associates, Inc. Publishers. Sunderland.

- **Tervala, H.L. & Antila, V. (1985).** Milk with anomalous renneting properties. *Meijeritieteellinen Aikakauskirja 43(1), 26-32.*

- **Trease, G. E. and Evans, W. C. (1989).** Pharmacognocy. *13thed. Balliere Tindall.*

- **Tsuruga, T., Chun, Y., Ebizuka, Y., and Sankawa, U. (1991).** Biologically active constituents of Melaleuca leucadendron: inhibitors of induced histamine release from rat mast cells. *Chem. Pharmcol. Bull., 39(12): 3276-3278.*

- **Tyrisevä, A.M., Elo, K., Kuusipuro, A., Vilva, V., Jänönen, I., Karjalainen, H., Ikonen, T. & Ojala, M. (2008).** Chromosomal regions underlying noncoagulation of milk in Finnish Ayrshire cows. *Genetics 180(2), 1211-1220.*

- **Tyrisevä, A.M., Ikonen, T. & Ojala, M. (2003).** Repeatability estimates for milk coagulation traits and non-coagulation of milk in Finnish Ayrshire cows. *Journal of Dairy Research 70(1), 91-98.*

- **Ulbricht, C., Abrams, T. R., Brigham, A., Ceurvels, J., Clubb, J., Curtiss,W., et al. (2010).** An evidence-based systematic review of rosemary (Rosmarinus officinalis) by the natural standard research collaboration. *Journal of Dietary Supplements, 7(4), 351e413.*

- **United States Department of Agriculture, Agricultural Research Services USDA. (2015).** National nutrient database for standard reference, release 25. Nutrient Data Laboratory. Retrieved from http://ndb.nal.usda.gov/ndb/foods/show/256.

- **Upson, T. (2006).** The board of trustees of the royal botanic gardens (Vol. 551, pp. 62-68). *Blackwell Publishing Ltd.*

- **Urech, E., Puhan, Z. & Schallibaum, M. (1999).** Changes in milk protein fraction as affected by subclinical mastitis. *Journal of Dairy Science 82(11), 2402-2411.*

- **Vallverdú-Queralt, A., Regueiro, J., Martínez-Huélamo, M., Rinaldi Alvarenga, J. F., Leal, L. N., & Lamuela-Raventos, R. M. (2014).** A comprehensive study on the phenolic profile of widely used culinary herbs and spices: Rosemary, thyme, oregano, cinnamon, cumin and bay. *Food Chem, 154, 299-307.* doi: 10.1016/j.foodchem.2013.12.106

- **Van Hekken, D. L. & Farkye, N. Y. (2003).** Hispanic cheeses: the quest for queso. *Food Technology, 57(1), 32-38.*

- **Vegara, S., Funes, L., Marti, N., Saura, D., Micol, V., Valero, M., (2011).** Bactericidal activities against pathogenic bacteria by selected constituents of plant extracts in carrot broth. *Food Chem. 128, 872–877.*

- **Walstra, P., Wouters, J.T.M. & Geurts, T.J. (2006).** *Dairy science and technology* New York: Taylor & Francis.

- **Wang, Q., Liu, C., Jing, Y.-p., Fan, S.-h., & Cai, J. (2019).** Evaluation of fermentation conditions to improve the sensory quality of broomcorn millet sour porridge. *Lwt, 104, 165-172.* doi: 10.1016/j.lwt.2019.01.037.

- **Wedholm, A., Larsen, L.B., Lindmark-Månsson, H., Karlsson, A.H. & Andrén, A. (2006b).** Effect of protein composition on the cheese-making properties of milk from individual dairy cows. *Journal of Dairy Science 89(9), 3296-3305.*

- **Williamson, E. M, Evans, F. J. and Wren, R. C. (1988).** Potter's New Cyclopedia of Botanical Drugs and Preparations. Saffron Walden (UK): C.W. Daniel Company Limited.

- **Xiao, J., Mao, F., Yang, F., Zhao, Y., Zhang, C., Yamamoto, K., (2011).** Interaction of dietary polyphenols with bovine milk proteins: molecular structure-affinity relationship and influencing bioactivity aspects. *Mol. Nutr. Food Res. 55, 1637–1645.*

- **Yamanoto, J.; Yamada, K.; Naemura, A.; Yamashita, T. and Arai, R. (2005).** Testing various herbs for antithrombotic effect. *Nutrition. 21(5): 580–587.*

- **Yang, H.L., Chen, S.C., Kumar, K.J.S., Yu, K.N., Chao, D.L., Tsai, S.Y., (2012).** Antioxidant and 645 anti-inflammatory potential of hesperetin metabolites obtained from Hesperitin- 646 administered rat serum: an ex vivo approach. *J. Agric. Food Chem. 60, 522–532.*

- **Yang, L., Jiang, J. G., Li, W. F., Chen, J., Wang, D. Y., & Zhu, L. (2009).** Optimum extraction process of polyphenols from the bark of Phyllanthus emblica L. based on the response surface methodology. *Journal of separation science, 32(9), 1437-1444.*

- **Yang, S.Y., Hong, C.O., Lee, G.P., Kim, C.T., Lee, W.W., (2013).** The hepatoprotection of caffeic acid and rosmarinic acid, major compounds of Perilla frutescens, against t-BHP-induced oxidative liver damage. *Food Chem. Toxicol. 55, 92–99.*

- **Yehb, M.H., Kaoc, S.T., Hunge, C.M., Liub, C.J., Leef, K.H., Yeha, C.C., (2009).** Hesperidin inhibited acetaldehyde-induced matrix metalloproteinase-9 gene expression in human hepatocellular carcinoma cells. *Toxicol. Lett. 184, 204–210.*

- **Yildirim-Elikoglu, S., & Erdem, Y. K. (2018).** Interactions between milk proteins and polyphenols: Binding mechanisms, related changes, and the future trends in the dairy industry. *Food reviews international*, *34*(7), *665-697.*

- **Ying Zhang, Lei Yang, Yuangang Zu, Xiaoqiang Chen, Fuji Wang, Fang Liu. (2010).** Oxidative stability of sunflower oil supplemented with carnosic acid compared with synthetic antioxidants during accelerated storage. *Food Chemistry. 118(3):656-662.*

- **Yu, H., Teng, L., Meng, Q., Li, Y., Sun, X., Lu, J., Teng, L. (2013).** Development of liposomal Ginsenoside Rg3: formulation optimization and evaluation of its anticancer effects. *International journal of pharmaceutics, 450(1-2), 250-258.*

- **Zahra, N. B., & Shinwari, Z. K. (2016).** What is done and what has to be done in Lamiaceae, a review of phylogenetics. *PeerJ Preprints, 4*, e2277v2271. *doi: 10.7287/peerj.preprints.2277v1*.

- **Zaouali, Y., Bouzaine, T., & Boussaid, M. (2010).** Essential oils composition in two Rosmarinus officinalis L. varieties and incidence for antimicrobial and antioxidant activities. *Food and Chemical Toxicology, 48, 3144e3152.*

- **Zeyneb Chaibeddra, Salah Akkal, Houria Ouled-Haddar, Artur M. S. Silva, Ammar Zellagui, Mohamed Sebti and Susana M. Cardoso (2020).** Scrophularia Tenuipes Coss and Durieu: Phytochemical Composition and Biological Activities. *Molecules 2020, 25, 1647; doi:10.3390/molecules25071647.*

- **Zhang, G., Hu, M., He, L., Fu, P., Wang, L., & Zhou, J. (2013).** Optimization of microwave-assisted enzymatic extraction of polyphenols from waste peanut shells and evaluation of its antioxidant and antibacterial activities in vitro. *Food and Bioproducts Processing, 91(2), 158-168.*

- **Zhao, J.; Zhu, W. H.; Hu, Q. and Guo, Y. Q. (2001).** Compact callus cluster and suspension cultures of *Catharanthus roseus* with enhanced indole alkaloid biosynthesis *in vitro. Cell Dev. Biol. Plant, 37: 68-72.*

- **Zhu, B. T.; Loder, D. P.; Cai, M. X.; Ho, C. T.; Huang, M. T. and Conney, A. H. (1998).** Dietary administration of an extract from rosemary leaves enhances the liver microsomal metabolism of endogenous estrogens and decreases their uterotropic action in CD-1 mice. *Carcinogenesis. 10: 1821-1827.*

Annexe 1:

Préparation des solutions pour le dosage des tannins

- Un gramme (1 g) de BSA dans un litre (1 L) de tampon acétate

Tampon acétate à pH = 5 { Acide acétique : 0,20 M ; Chlorure de sodium : 0,17 M ; pH ajusté à 5

- Pour la solution SDS/TEA : Mettre 50 ml de TEA et 10g de SDS dans une fiole de 1000 ; ajuster au trait de jaugé avec l'eau distillée en agitation continue (pour éviter d'avoir des bulles de détergent qui vont fausser l'ajustement).

Annexe 2:

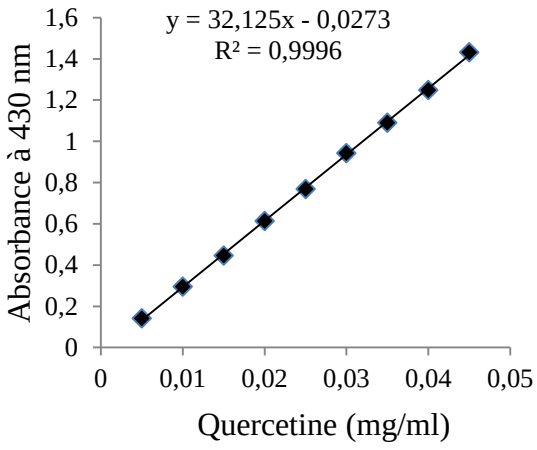


Figure 2: Courbe d'étalonnage pour le dosage des flavonoïdes.

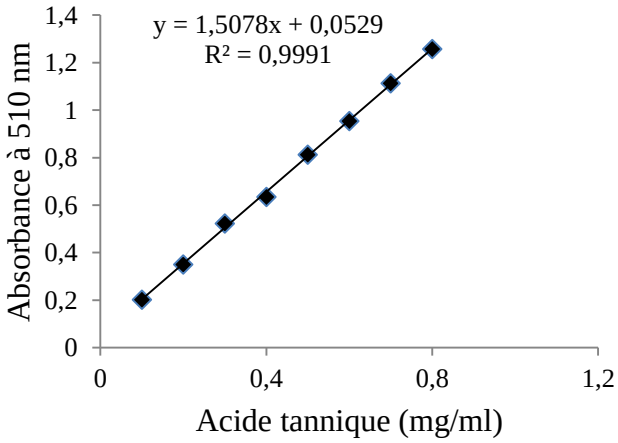


Figure 3: Courbe d'étalonnage pour le dosage des tannins.

Annexe 3:

Composition des milieux préparés

Gélose nutritive

Peptone……	10 g
Extrait de viande…….	5 g
Chlorure de sodium…….	5 g
Agar……..	15 g
	pH = 7,2

Bouillon nutritif

Peptone……..	10 g
Extrait de viande……..	5 g
Chlorure de sodium……..	5 g
	pH= 7,2

Eau physiologique

Chlorure de sodium…….....	9 g/l

Répartir dans les tubes à essais, les mettre dans un autoclave pendant 20 minutes à 120°C.

- Les milieux de culture (MRS et M17): Ces milieux sont sous forme déshydratée. Leur préparation se fait en suivant le mode opératoire indiqué sur chaque boite puis ils sont répartis dans des tubes ou flacons. Ces milieux, une fois préparés, ils sont autoclavés à 121°C pendant 15minutes afin de les stériliser.