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Formulation d'un aliment (fromage) à valeur ajoutée

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List of abbreviations:

AN	Algerians Norms
ANOVA	Analysis of variance
°C	Celsius degree
C. C	Control cheese
EU	European Union
EO	Essential Oil
FAO	Food and Agriculture Organization
FDM	Fat in dry matter
G	Gram
H°	Moisture or Humidity
ISO	International Organization of Standardisation
IU	International Unit
Kcal	Kilocalories
MAE	Microwave assisted extraction
Min	Minute
N°	Number
PDM	Protein in dry matter
pH	Hydrogen Potential
%	Percentage
SIM	Salt in moisture
T°	Temperature
UK	Union kingdom

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Introduction

Milk, because of its great nutritional qualities, has always been considered as a food in its own right, but its consumption has often been limited because of its great instability. The irregularity of the production, by its seasonal character, and the great fragility of the product encouraged the producers to seek forms of rest of the essential elements of milk**.** It is in this context that the first cheese transformations appeared several millennia ago. The man quickly realized that the destabilization of milk facilitates the expulsion of water and creates conditions favorable to the conservation **(Jeantet, 2017).**

Cheese was originally a way of preserving milk, or at least the elements that could be preserved through fermentations that man learned to direct **(Eck and Gillis, 2006)**. Depending on the type and nature of the milk used and the manufacturing technologies implemented, several varieties of cheese have appeared **(Guiraud, 2003)**. Among them, Halloumi cheese, which is of Cypriot origin, has a peculiarity that distinguishes it from other cheeses "Halloumi has the ability to be cooked at high temperatures without melting **(Mehanni, 2020)**

This study aims to develop a Halloumi cheese with added value (enriched by rosemary), in order to procure for the cheese, the beneficial properties of this plant and thus improve its organoleptic quality namely the aromatic property.

It is in this context that the objective of our work is to develop a new cheese fortified with rosemary plant (powder, leaves and essential oil), using un experimental design. The aim of this research is to evaluate the effect of the incorporation of this plant in the elaborated cheese and to study its physicochemical and microbiological characteristics.

This work is composed of two main parts:

- ✓ The first part intended for the bibliographic synthesis: overview of milk, cheese and rosemary.
- ✓ The second experimental part which includes two chapters: one on the description of the stages of formulation of the cheeses (enrichment cheese, as well as methodologies of physicochemical and microbiological analyses, and a second chapter presenting the interpretations of all the obtained results. This study ends with a conclusion.

Bibliography

Chapter I:

Overview of milk and cheese

I.1. Overview of milk

I.1.1. Definitions

Milk is the product of secretion of the mammary glands of the mammals, (cow, goat and ewe), intended for the food of the young incipient animal **(Hui Y-H, 1992).**

According to the **codex Alimentarius**, Milk is the normal mammary secretion of milking animals obtained from one or more milking without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing.

Milk was defined in **1908**, during the **International congress of the Repression of the frauds** in Geneva as being: "The integral product of the total and uninterrupted draft of a quite bearing well-nourished and not overworked dairy female. Milk must be collected properly and does not have to contain colostrum" **(Alais, 1975).**

I.1.2. Composition of milk

Milk consists of protein (casein and whey proteins), lipid, lactose, minerals (soluble and insoluble), minor components (enzymes, free amino acids, peptides) and water **(Tab.I)**. The casein fraction coexists with insoluble minerals as a calcium phosphate-casein complex. The water and its soluble constituents (lactose, native whey proteins, some minerals, citric acid….) are referred to as serum (**Walther et al., 2008)**

Table I. Milk average composition of various animal species **(Carole and Vignola, 2002)**

Animals	Moisture (%)	Fat (%)	Proteins (%)	Carbohydrate (%)	Minerals (%)
Cow	87.5	3.7	3.2	4.6	0.8
Shepp	81.5	7.4	5.3	4.8	1.0
Goat	87.0	3.8	2.9	4.4	0.9
Camel	87.6	5.4	3.0	3.3	0.7
Mare	88.9	1.9	2.5	6.2	0.5

I.1.3. Physicochemical properties of milk

The main physico-chemical properties used in the dairy industry are the following:

- Density: The density generally expressed in g/ml or kg/l, is a physical property which varies according to the temperature (T°). The density of milk at 15°C varies from 1.028 to 1.035 for an average of 1.032 **(Carole and Vignola, 2002).** Two opposite factors of variation determine the density: concentration of the dissolved elements and in suspension (solid not fat) and the proportion of the fat content **(Mathieu, 1998).**

- Freezing point: The freezing point of milk is slightly lower than that of water since the presence of solubilized solids lowers the freezing point. It can vary from -0.530°C to -0.575°C with an average at -0.555°C. The freezing point is checked with a cryoscope **(Carole and Vignola, 2002).**

- Boiling point: The boiling point is the temperature reached when the vapor pressure of the solution is equal to the applied pressure. It is slightly higher than the boiling point of water, which is 100.5°C **(Carole and Vignola, 2002).** Thus, as for the freezing point, the boiling point is influenced by the presence of solubilized solids

- Titratable acidity or Dornic acidity: As soon as it leaves the cow's udder, the milk has certain acidity. This acidity is mainly due to the presence of proteins, especially caseins and lactalbumins, mineral substances such as phosphates and carbon dioxide, and organic acids, most often citric acid. **(Carole and Vignola, 2002).** A normal fresh milk has a titratable acidity of 16 to 18 degrees Dornic; 16 to 18 in decigrams of lactic acid per liter (**Veisseyre, 1975)**

- pH: The pH of fresh milk is between 6.6 and 6.8. Unlike Dornic acidity, pH does not measure the concentration of acidic compounds but rather the concentration of H+ ions in solution. The pH values represent the state of freshness of milk, especially with regard to its stability, because it is the pH that influences the solubility of proteins, that is to say, reaching the isoelectric point. **(Carole and Vignola, 2002)**

I.1.4. Organoleptic properties

The main organoleptic characteristics of milk are given in table II.

Table II. Organoleptic characteristics of milk **(Veisseyre, 1975).**

Attributes	**Characteristics**
Color	Yellowish-white to matte-white (due to reflection of light on micelles and caseins). Bluish or yellowish (milk rich in lactoflavin).
Taste	Slightly accentuated, depending on the species and the food
Odor	Slightly sweet (lactose has a low sweetening power)
Viscosity	Twice as viscous as water: - more viscous in monogastrics than in polygastrics - more viscous at the beginning of lactation (colostrum)

I.1.5. Nutritional value of milk

Milk is the most complete food known in its natural state because it contains significant amounts of the 55 or so nutrients essential for life. With regard to its content of metabolizable energy, milk has a high concentration of nutrients; it is therefore considered a food of high nutritional value. However, milk is not a perfect food because it does not naturally contain fiber and its composition in certain nutrients, such as iron and vitamin D, is relatively low. **(Amiot et *al*., 2002).** Milk is a liquid food, but its dry matter content (10 to 13%) is close to many solid foods. Its energy value is 700 Kcal/L, its proteins have a high nutritional value, especially lactalbumin and lactoglobulin**.** Milk is a source of calcium and phosphorus, but it contains little iron, copper, ascorbic acid, niacin and vitamin D **(Kodio, 2005).**

I.2- Overview of cheese

I.2.1. History and definition

Not only can milk be consumed in its natural state, it can also undergo various biotransformations that contribute to considerably broaden its nutritional and sensory qualities **(Carole and Vigonla, 2002).** The word "cheese" comes from the Latin "formaticum" which means "that which is made in a form". In the middle Ages (1200-1500 A.D.), the word used was fromgi, fromton which means variation or forming **(Dulor, 2002).**

Cheese is an ancestral form of preservation of the milk constituents and has a high nutritional and epicurean interest. At the present time, these products have very different shapes and tastes depending on the origin of the milk (cow, sheep, goat, etc.) and the applied technology. Thus, more than 1000 varieties of cheese have been identified. The origin of the cheese goes back to the highest antiquity, the first cheeses weremade in western Asia 8000 years ago. **(Carole and Vignola, 2002).** Although milk is virtually the same around the world, the diversity of cheese textures, tastes and aromas is almost infinite, and virtually any cheese can be made anywhere in the world. The nuances of texture and taste, however, are determined by the raw material: the type and breed of animal, the soil, the pasture, the climate, the microclimate, and the skill of the cheesemaker **(Harbutt, 2010).**

The FAO/WHO Codex Alimentarius Standard No. A6 (1996) defines cheese as "a fresh or ripened product of solid or semi-solid consistency in which the ratio of serum protein to casein does not exceed that of milk and obtained by complete or partial coagulation of the following raw materials: milk, skimmed milk, partly skimmed milk, cream, or buttermilk, alone or in combination, through the action of rennet or other suitable coagulating agents and by the partial draining of the whey resulting from this coagulation"

I.2.2.Composition and classification

Cheese is very rich in its composition, in proteins, water, bioactive peptides, amino acids, lipids, fatty acids, vitamins and minerals **(Tab.III**) **(Walther et al., 2008).**

Table III. The average composition of different types of cheese.

Constituents	Fresh cheese	Soft cheese	Processed cheese
Moisture (g)	80	50	48
Carbohydrates (g)	4	4	2.5
Fat (g)	7.5	24	22
Protein (g)	8.5	20	18
Calcium (mg)	100	400	680
Sodium (mg)	40	700	1650
Vitamin A (IU)	170	1010	1200

IU: International Unit

- The diversity of cheese makes their classification difficult. The latter is all the more complicated to establish as the characters on which a classification is based are intermingled **(Mietton, 1994).**

- **Official classification:** According to the international standard FAO / WHO No. A-6 the official classification of cheese is illustrated in **Appendix 1.**
- **Didactic classification: Lenoir et al (1983)** give a synthetic and didactic view of the diversity of the cheese according to the modalities of coagulation, draining and maturation of the curd, hence the great diversity of the cheese **(Appendix 2).**

I.2.3.Nutritional value

One of the main components of cheese is fat, which represent 20 to 30% of the total dry matter and contribute to the flavor of fresh or ripened cheese **(Walther & al., 2008).** Cheeses are also rich in proteins containing essential amino acids **(Scott & al., 1998)**, so 100g of fresh cheese provides 30% to 40% of the daily protein requirement for an adult, while an equivalent amount of hard cheese provides 40% to 50% **(Renner, 1987).** Cheese contains appreciable quantities of minerals, with iron, calcium and phosphorus being the most abundant. Indeed, 100g of hard cheese can supply 50% of the daily phosphorus needs of an adult **(Tsuchita et al., 2001).**

I.2.4. HALLOUMI Cheese

I.2.4.1 Definition and history

Traditional Halloumi cheese **(Fig.01)**, which is of Cypriot origin, is semi hard, elastic, has no obvious skin/rind and the texture is close with no holes and it is easily slicedand preserved in brine**(Papademas and Robinson, 1998).** Its color varies from white (when ovine or caprine milk is used) to yellowish (when bovine milk is the main ingredient). It can be consumed raw, but it is usually grilled, fried or grated over a hot dish. This cheese is also extremely popular in the Middle East and the Mediterranean regions **(Papademas and Robinson, 1998).**Halloumi cheese, as it is known in southern Cyprus and Hellim cheese in northern Cyprus. This cheese, often grilled on the barbecue is made in the north as in the south **(Garanti, 2016)**

Figure 01: HALLOUMI cheese

Cheese making has been practiced for several thousand years, mainly to preserve the milk. Many cheese varieties that are made in the Middle East probably originated from these early products, and Halloumi may well be one of them. Although it was originally popular only in Cyprus, its appeal has spread around the world, and exports to the European Union (EU). The United Kingdom (UK) absorbs more than 60% of Cyprus' total imports to the EU each year **(Papademas and Robinson, 1998).**

As for the historical root and origins of hellim / Halloumi cheese, there is no consensus on the issue. While some insist that it is exclusively Greek Cypriot or Turkish delicacy, etymologically, the term Halloumi has an Arabic root and cultural historians point to Venetian sources, which had encountered Halloumi in the pre-Ottoman period. The study conducted by **Osam and Kasapoglu (2010)** revealed that

in historical and archaeological studies, the origin of Halloumi goes to the Egyptian and Roman civilizations.

I.2.4.2. Composition of Halloumi cheese

According to Cypriot standards, Halloumi cheese should contain a maximum of sodium chloride at 3%, have a minimum fat content of 43% dry matter, and a maximum moisture content of 46% (**Tab.VI**). However, these figures apply mainly to traditional Halloumi cheese (goat's milk and sheep's milk), unlike that produced from cow's milk, generating a different chemical composition **(Papademas and Robinson, 2000).**

Table IV. Physico-chemical characteristics of Halloumi cheese made from milk with different fat contents **(Theophilou and Wilbey, 2007)**

Milk											
	1	**2**	**3**	**4***!	**5***	**6***	**7***	**8**	**9 !**	**10***	**11**
Weight (kg)	32	29,7	30,6	29	35	30	30	31	31	31	31
Fat (%)	1,61	3,45	2,69	2,16	3,82	3,13	2,10	4,04	2,40	2,40	3,10
Proteins (%)	3,36	3,33	3,34	3,57	3,40	3,47	3,52	3,50	3,80	3,50	3,40
pH	6,5	6,5	6,5	6,29	6,46	6,29	6,31	6,6	6,7	6,7	6,7
Cheese											
Weight (kg)	2.35	2.69	2.90	2.68	2.89	2.29	2.22	3.60	3.05	2.79	2.89
Yield (%)	7.4	9.01	9.5	9.2	8.3	7.6	7.4	11.6	9.8	**9**	9.3
Protein (%)	32.3	24.1	22.0	28.6	21.5	23.4	30.0	17.8	24.4	25.6	23.2
Fat (%)	18	27	21	21	28	25	21	27	20	22	25
Salt (%)	1.93	2.14	2.75	2.35	2.13	1.72	1.73	2.71	2.44	2.31	2.22
Moisture (%)	44.5	43.3	49.7	47.84	47.95	47.95	44.98	49.04	48.76	46.71	45.84
S/M (%)	4.3	4.9	5.5	4.9	4.4	3.6	3.8	5.5	5.0	4.9	4.8
PDM (%)	58	43	44	55	41	45	55	35	48	48	43
FDM (%)	32	48	42	40	54	48	38	53	39	41	46
pH	6.65	6.63	6.50	5.54	5.52	5.53	5.46	6.69	6.70	6.40	6.73
P/M (%)	73	56	44	60	45	49	67	36	50	55	51
Meltability (%)	35	208	179	375	507	690	460	237	137	175	172

S/M : Salt in Moisture ; **PDM :** Protein in Dry Matter ; **FDM :** Fat in Dry Matter ; **P/M :** Protein in Moisture, *Started culture added**,** !4 and 9 contained microparticulted whey protein

I.2.4.3. HALLOUMI cheese manufacturing

During cheese manufacture, the milk is subjected to a partial dehydration involving controlled expulsion of serum and concentration of fat, casein (and in some

cases denatured, aggregated whey proteins) and some of the minerals. **(Eck and Gillis, 2006).** The manufacturing of Halloumi depends on the localities in Cyprus, and this situation creates a lot of confusion as to which procedure is correct, thus it has been evaluated in several trials that is now governed by the regulations of government agencies). Halloumi cheese is traditionally made from sheep's milk, goat's milk or a mixture of both. More recently, some brands have come onto the market with cow's milk as the main ingredient (**Fig.02**). **(Papademas and Robinson, 1998)**

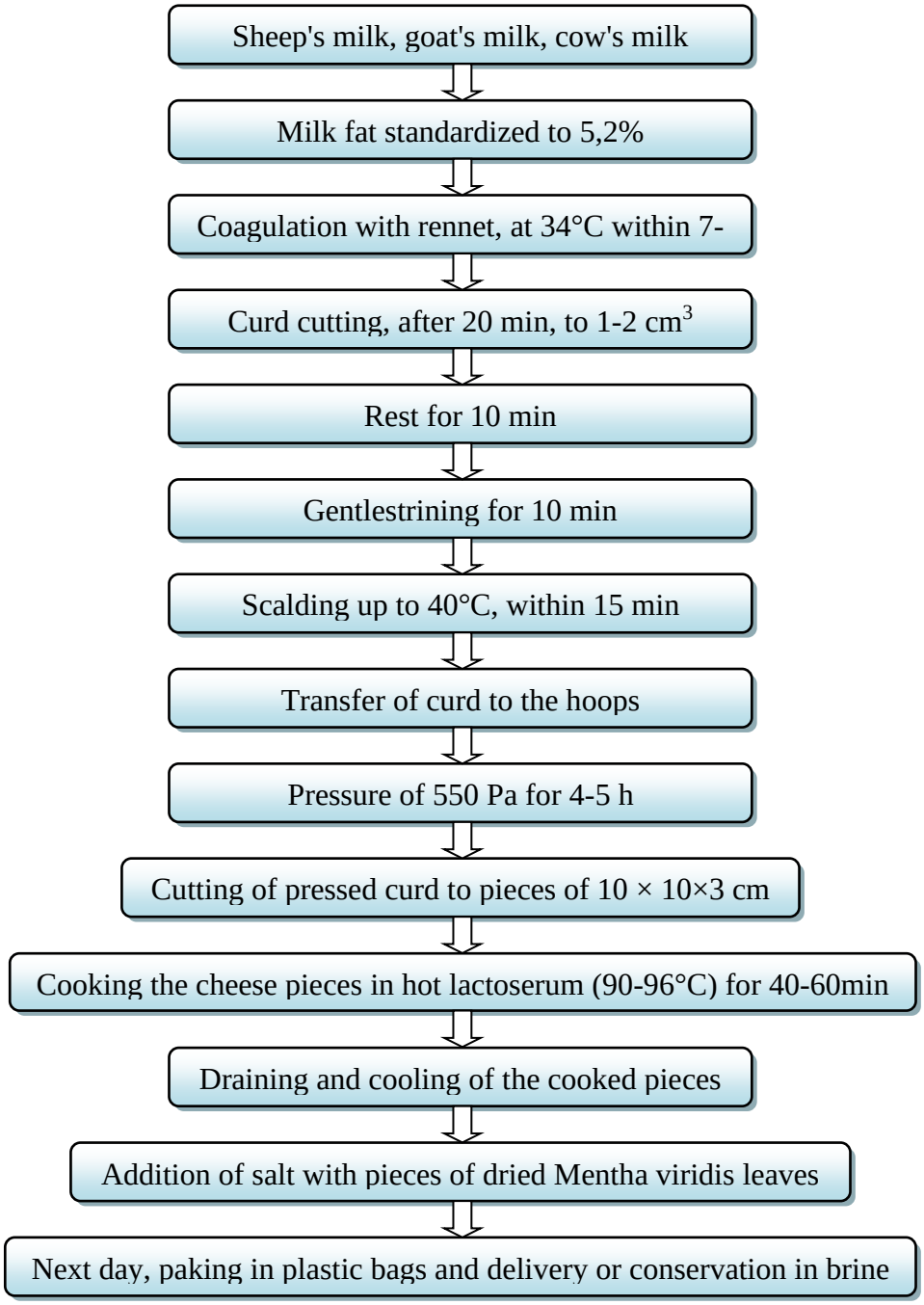

Figure 02: Diagram of HALLOUMI cheese production **(Kaminarides and al, 2015)**

- **Culinary uses of Halloumi cheese**

Halloumi cheese can be eaten raw, but it is usually grilled, fried or grated on a hot dish (**Fig.03**). This cheese has a specific characteristic, it can be cooked at high temperature, without melting. It stretches on heating, which means that the compact texture of the cheese will be partly lost and a degree of flow introduced into the melted cheese.These characteristics are often referred to as the mutability and stretchability of cheese. It accompanies all kinds of food: salads, grilled meat and sausages, eggs and many other foods because of its delicious flavor. **(Papademas and Robinson, 1998)**

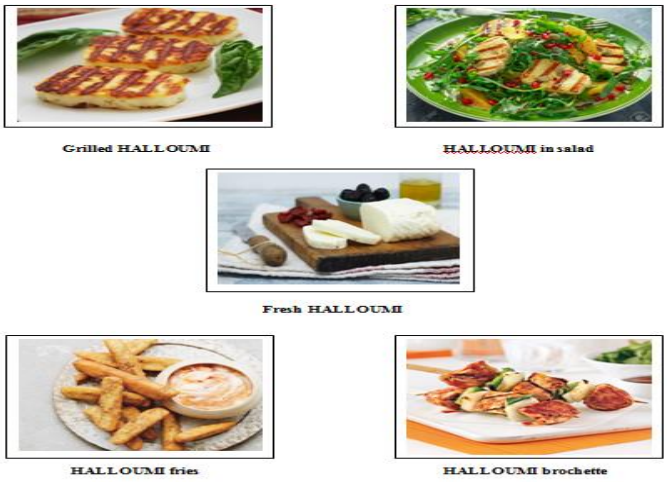

Figure 03: Different culinary uses of HALLOUMI cheese

Chapter II:

Overview of rosemary

II. Overview of rosemary

II.1. Description

Rosemary *(Rosmarinus officinalis*) is a plant of the family Lamiaceae growing spontaneously on the Mediterranean (**Fig.04**). It can be found as a shrub, under shrub or herbaceous, always green with elongated leaves and blue to mauve flowers measuring about 0.6 to 1.8 m high.Rosemary takes its name from the Latin, ros, from dew and marinus, from sea: allusion to its perfume, its pungent taste and its habitat on the sea-sides. **(Fadili1 et al., 2015).**

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae
Genus	Rosmarinus
Species	*Rosmarinus officinalis L.*

Figure 04: Taxonomic classification of rosemary **(Gaussen et al., 1982).**

- Rosemary exists in arid and dry regions, low and rocky hills and mountains, on limestone, shale and clay soils **(Gaussen et al., 1982).** It is a perennial, shrubby plant, native to the Mediterranean basin and southwestern Asia, nowadays spread almost everywhere in temperate climates with mild winters. The plant likes full sun and is moderately drought tolerant **(Bousbia, 2011).** There are several species of rosemary in the world: *R. officinalis*, *R.eriocalyx* and *R. lavandulaceus.* It is cultivated from the beginning of September until summer **(Wichtl and Anton, 2003).**

II.2. Chemical composition

A wide variety of useful secondary metabolites has been isolated from rosemary plants, including essential oil (EO) and phenolic compound **(Ribeiro -Santos & al., 2015)**. However, the accumulation of bioactive compounds depends on many factors, such as climatic conditions, variety, plant part, extraction technique…..etc **(Ribeiro -Santos et al., 2015)**. This plant includes, in addition to the mineral element, lipids, sugar and high levels of vitamins **(Orhan et al., 2008; Švarc-Gajić et al., 2013).**

Table V: Chemical properties and mineral contents of rosemary **(Švarc-Gajić et al., 2013)**

Fraction	Content (100g)	Element	Content (mg/kg)
Total lipid (g)	67,7	**Calcium**	7792
Sugar (g)	20,7	**Magnesium**	1635
Fiber (g)	14,1	**Phosphorus**	1475
Vitamine A (I.U)	2924	**Iron**	330
Vitamine C (mg)	21,8	**Sodium**	2712
Riboflavine (mg)	0,152	**Potassium**	14916

II.2.1. Phenolic composition

Phenolic compounds (polyphenols) are products of the secondary metabolism of plants, widely distributed in nature, having several phenolic groups **(Bahorun, 1997).**

a- Phenolics acids:

There are two main classes of phenolic acids **(Fig.05)**, benzoid acid (C1-C6) derivatives and cinnamic acid derivatives (C3-C6). The main phenolic acids of rosemary are rosmarinic acid, vanillic acid, caffeic acid, gallic acid and p-coumaric acid. **(Pereira et al., 2017)**

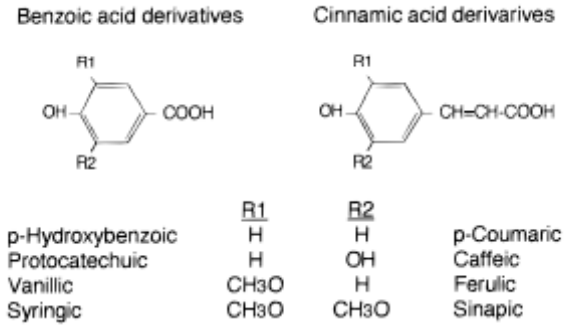

Figure 05: Chemical structure of benzoic acid and cinnamic acid derivatives **(Natella et al., 1999)**

b- Flavonoids:

The term flavonoids cover a wide range of natural compounds, they are mainly responsible for the pigmentation of plants. Favonoids have a common biosynthetic origin and they all have the same basic sketlon **(Fig.06)**, fifteen carbon atoms composed of two aromatic units, C6 cycle (A and B), linked by a C3 chain **(Crozier, 2003).**

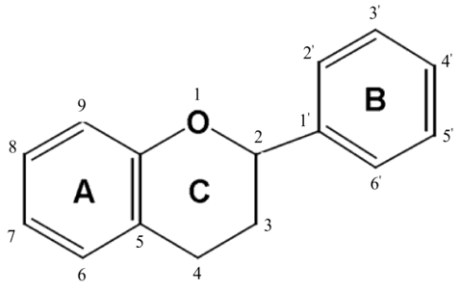

Figure 06: General structure of flavonoid **(Alkhalidy et al., 2018)**

- According to **Borras-Linares et al (2015)**, the main flavonoid identified in rosemary is mainly flavonoic derivatives (luteolin and quercetin derivatives).

c- Tannins:

Tannins are complex phenolic compounds obtained from condensation of simple phenols. They are divided into two groups **(Fig.07)**: hydrolysable tannins (carbohydrate ester and phenolic acids) and condensed tannins (dimmers oligomers, oligomers and/or polymers of favannes-3-ols or flavannes-3, 4-diols). A Study has shown that rosemary extract contains Gallic tannins **(Fadili et al., 2015).**

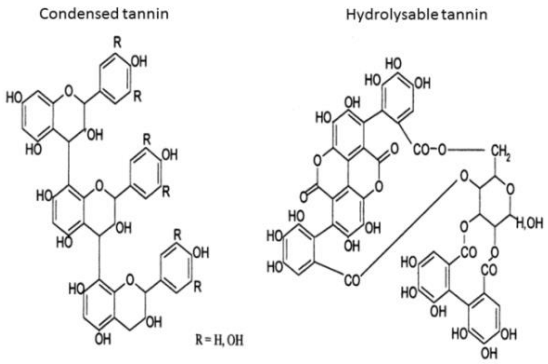

Figure 07: Chemical structure of tannins **(Janna Moat, 2016)**

II.2.2. Essential oils

Essential oils are odiferous, highly volatile substances, produced as secondary metabolites in plants; EOs can be obtained by means of water distillation, water and steam distillation, or steam distillation alone **(Ramos et al., 2015).** EO can vary according to temperature, soil conditions, altitude, the country of origin and the part of the plant **(Mariod, 2016).** EO have a complex composition, characterized by hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols and phenol ethers) **(Nerio et al., 2010).**

Rosemary is relatively rich in essential oils (1 to 5%). Consisting of several chemical molecules of natural synthesis. These molecules are different depending on the nature of the plant and the soil on which it is grown **(Bousbia, 2011).** According to **Ribeiro-Santos (2015)**, the predominant constituent of rosemary essential oils is cineole (50.2%).

II.3. Pharmacological activities

Rosemary is a well-known and considerably valued medicinal plant, widely used in pharmaceuticals and traditional medicine. It is known for its antioxidant, antimicrobial, analgesic, anti-inflammatory and antiulcerogenic properties **(EL Kamli et al., 2017).** Its aerial parts are used orally, in traditional medicine, to relieve dysmenorrhea and renal colic and as an antispasmodic **(Gonzalez-Trujano et al., 2007**).

R. officinalis L. can promote several pharmacological effects due to the interaction between the molecules of the plant and the organic systems. The effects demonstrated by this plant include ability to attenuate asthma, atherosclerosis, cataract, renal colic, hepatotoxicity, peptic ulcer, inflammatory diseases, ischemic heart disease; antioxidant and anti-inflammatory actions of rosmarinic acid ; control of hypercholesterolemia and oxidative stress and relief of physical; myocardial blood pressure reduction with rosmarinic acid; antiulcer action; lipid peroxidation reduction in heart and brain; antiangiogenic and neuro-protective effects of carnosic acid and carnosol; prevention of problems related to atherosclerosis; anticancer and antiproliferative effects; antiviral; and antimicrobial actions; hepato protective, nephro protective and radio protective-antimutagenic capacities; glycemia reduction; muscle relaxant and treatment for cutaneous allergy; ability to treat depressive behavior **(De Oliveira et al., 2019).**

☞ **Antioxidant activity of rosemary:**

Antioxidants play a major role in the prevention and treatment of diseases associated with oxidative damage, including cancer, cardiovascular and neurodegenerative diseases. Reactive oxygen species, including hydrogen peroxide and free radicals, such as superoxide anion (O•-) and hydroxyl radical (HO•), are inevitably produced in living organisms resulting from metabolic processes or from external sources. Several *in vitro* studies were reviewed regarding the antioxidant activity of the main isolated compounds from rosemary, namely carnosic acid and rosmarinic acid. These bioactive compounds and the essential oil were validated for their antioxidant activity. Also, using the lipid free radicals scavenging activity assays and Rancimat methods (determination of oxidative stability of fat), the bioactive rosemary compounds, has been reported to inhibit lipid peroxidation through the lipid free radical scavenging mechanism **(Andrade et al., 2018)**. *R Officinalis* leaves are commonly used as a condiment for flavoring food, and as a source of antioxidant compounds employed in food conservation **(De Oliveira et al., 2019).**

- Table VI present examples of some works of enrichment of cheese with antioxidants

Table VI: Enrichment of cheese with antioxidants

Type of cheese	**Matrix**	**Compound**	**Experimentsonditions**	**Reference**
Sheep cheese	Chia oil	Omega 3	-Preparation of chia oil emulsion : 30 g of chia oil and 70g of calcium caseinate suspension ; -The mixture was emulsified at 40°C at 7000 rpm for 2 min. Enrichment: -3g/l : α-linoleic acid increased from 34.79 to 262.78 -5g/l :α-linoleic acid increased from 34.79 to 536.43.	**(Muñoz et al., 2019)**

Cheddar	Green tea extract (GTE) ;powder	Phenolic compound	- 1 and 2 gof GTE/kg of milk Antiradical activity of control cheese was 20, 8 mM Trolox Eq.g^{-1}, after 15 days: increment ($P \leq 0.05$) of this activity to 25, 1 and 27, 6 mM Trolox Eq.g^{-1}. -After 29 days of storage: reduction ($P \leq 0.05$) by 25% in the control cheese and by 18% in the cheese containing GTE at a concentration of 1 g.kg−1 of milk, but antiradical activity persisted in cheese containing GTE at a concentration of 2 g.kg−1 of milk.	**(Giroux et al., 2013)**
Feta	Peppermint extract (PE)	Antioxidant	220 - 660µg PE/g of cheese Better antioxidant activity is recorded at a concentration ofPE 227ug/g of cheese	**(Fadavi et Beglaryan, 2015)**
Gouda	Mango (*Mangifera indica* L.) kernel fat		- Extraction solid-liquide (Hexane) - Total phenolic content increased 19 times over the control Feta cheese. - Total flavonoid content increased 37 tiles over the control Feta cheese.	**(Kahn et al., 2018)**
Fresh cheese	Rosemary leaves powder	Phenolic compounds	Microwave –assisted extraction	**(Himed-Idir et al., 2021)**

Experimental part

Chapter III: Materials and Methods

III.1. The experimental design in cheese preparation

Before proceeding to the manufacture of cheese Halloumi, a design of experiment was carried out to optimize the conditions of its preparation. First, a preliminary study was conducted to determine the minimum and maximum values of each component of the plan. These values will be introduced in the software (JMP 9) in order to generate an experimental plan to determine the optimal mixture of the raw materials that will allow obtaining a cheese with a good yield and better organoleptic qualities.

This software is used to obtain a maximum of information with a minimum of experiments.

III.1.1. Preliminary study

In the preparation of Halloumi cheese, to determine the limit of the experimental design, a set of parameters (amount of cow's milk, goat's milk and milk powder) were studied, therefore, a preliminary study is requested.

This present work is carried out to complete the preliminary study that was conducted by **Hamdaoui et al., (2018);** they are the first who proceeded to the preparation of Halloumi cheese at the University of Bejaia. During their study preliminary tests were conducted by preparing 4 types of cheese by varying two parameters: the amount of cow's milk and that of goat's milk and setting the other preparation parameters (amount of milk powder, amount of rennet, pressing time ...). The cheeses that were prepared in their study are the following:

- Cheese 1: prepared with 100% pasteurized cow's milk and 3% milk powder.
- Cheese 2: prepared with 80% pasteurized cow's milk, 20% goat's milk and 3% milk powder
- Cheese 3: prepared with 60% pasteurized cow's milk and 40% goat's milk and 3% milk powder.
- Cheese 4: prepared with 80% pasteurized cow's milk and 20% goat's milk and 3% powdered milk. It is the same composition as cheese 2 but cheese 4 is seasoned with nigella seeds.

Sensory and hedonic analyses were carried out to describe the organoleptic characteristics of these 4 cheeses and to classify them by order of consumer preference.

III.1.2. Design of experiment

An experimental design was conducted using 'STATISTICALS DISCOVRY FROM SAS JMP' to optimize the optimal proportions of the three components in the formulation of cheese Halloumi: content of cow's milk, content of goat's milk and milk powder. The minimum and maximum limits of each of these three parameters were set based on the results of the preliminary study; however the other preparation parameters remain unchanged. The plan obtained consists of 15 formulas of Halloumi cheese, by respecting the same conditions and proportions indicated in the following table:

Table VII. Experimental design for the preparation of Halloumi cheese

Cheese	cow's milk	goat's milk	milk powder
N°1	800	600	60
N°2	600	400	60
N°3	600	200	40
N°4	400	200	60
N°5	600	400	60
N°6	600	400	60
N°7	400	400	40
N°8	800	200	60
N°9	600	600	80
N°10	800	400	80
N°11	600	200	80
N°12	800	400	40
N°13	400	600	60
N°14	400	400	80
N°15	600	600	40

III.1.3. Validation of the experimental design and selection of the optimum

In order to validate the experimental design by adding responses to the software JMP and obtain an optimal formula, calculations of performance and sensory and hedonic analysis were performed for the 15 samples of cheese that were prepared.

The sensory analysis were carried out at the laboratory of sensory analysis of the University of Bejaia, which we used a panel of 10 judges to evaluate the organoleptic characteristics of each cheese sample. For this reason, 3 sessions were scheduled to analyze the 15 cheeses; in each session 5 samples were analyzed.

The different parameters evaluated were: cheese yield, color, smell, aroma, taste, texture and preference. Scores between 1 and 5 were attributed according to the intensity of the organoleptic characteristics evaluated. Preference scores (product appreciation) were given on a scale of 9, as shown in the questionnaire distributed to the experts **(Appendix 3, 4 and 5).**

III.2. Preparation of Halloumi cheese

The preparation of the cheese was carried out in the sensory analysis laboratory, following the manufacturing steps described by **Papademas et Robinson (1998)**.

- Raw materials used (Fig.08):

- ✓ Pasteurized cow's milk manufactured by (the dairy industry of Amizour);
- ✓ Raw goat milk is purchased from a farm in Bejaia;
- ✓ Milk powder from local market;
- ✓ Rennet powder from dairy industry Soummam (CHY-MAX Powder Extra NB)

- Manufacturing steps:

C.T 60%: prepared with 60% goat milk, 40% pasteurized cow milk and milk powder;

C. OE 60%: prepared with 60% goat milk, 40% pasteurized cow milk and milk powder + 0.01 % of rosemary essential oil (0.1%);

C. P 60%: prepared with 60% goat milk, 40% pasteurized cow milk and milk powder + 0.5 % of rosemary powder;

C. L 60%: prepared with 60% goat milk, 40% pasteurized cow milk and milk powder + 3% of rosemary leaves.

III.2.1. Reception and filtration of milk

Upon receipt of the milk, it is filtered through a sieve to remove physical impurities and then measured of pH to ensure the freshness of the milk. Thus it is kept cold until used.

Figure 08: Photography of cow's, goat and powder milk

III.2.2. Preparation of milk mixtures and heat treatment

The manufacture of this cheese is the preparation of one liter of milk mixture according to the type of cheese to be prepared. Followed by the heat treatment (at 90°C), which aims to destroy the pathogenic bacteria present in vegetative form and vegetative form and to reduce the total flora **(Luquet, 1990; Carole and Vignola, 2002).**

Figure 09: Photography of heat treatment

III.2.3. Precipitation and coagulation

After the milk was cooled to about 30-34°C, 2 mL of 0,5 g/L liquid rennet was added, followed by a good stirring. A firm curd was obtained after 45 min of rest. Coagulation corresponds to a physicochemical modification of the casein micelles under the action of the action of proteolytic enzymes (rennet). These lead to the formation of a three-dimensional protein network called coagulum or gel, which then allows the expulsion of a water and soluble matter **(Eck and Gillis, 2006)**.

Figure 10: Photography of step of coagulation

III.2.4.Cutting the coagulum

At this stage, the curd is cut with a knife into cubes (1-2 cm^3) to facilitate the evacuation of the whey. It is then heated to 38 to 40°C for 20 minutes under a gentle to increase the firmness of the curd and accelerate the release of the whey **(Papademas and Robinson, 1998)**.

Figure 11: Photography of coagulum cutting

III.2.5. Filtration and draining

In this stage the curd is recovered after filtration using a strainer and a fine cloth. The draining of the curd is done manually with a light pressing, it allows the progressive elimination of the remaining whey. The recovered whey is kept in order to use it during the cooking stage. The draining leads to a mass of curd whose dry extract is more or less concentrated. This physical phenomenon of separation of the dispersing phase is called

syneresis **(Eck and Gillis, 2006)**. During this stage, most of the soluble elements are eliminated in the whey **(Carole and Vignola, 2002).**

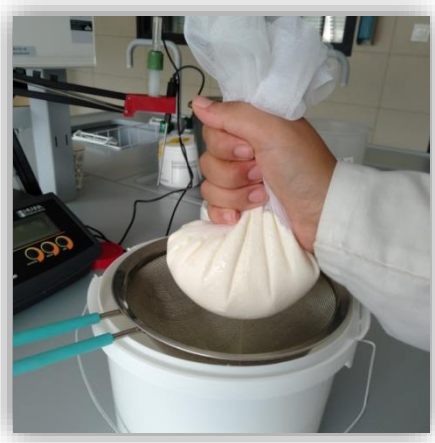

Figure 12: Photography of filtration and draining

III.2.6. Molding and pressing

This step gives the cheese its shape and continues the elimination of the whey. For this purpose, a pressure of 550 Pa is applied using a weightinstead of a hydraulic press. It is applied for 4-5 hours which allows thefree water from the cheese paste, thus completing the draining process and allowing the curd grains toallow the curd grains to fuse together and form the semi-firm cheese paste **(Papademas and Robinson, 1998)**.

III.2.7. Cooking of the cheese paste

Firstly, the whey removed from the curd is heated with gentle agitation between 80-82°C, to coagulate the remaining whey proteins. The whey is then filtered to recover the soft mass that can be used for the production of fresh cheese **(Raphaelides et al., 2006).**

Then the cheese paste is removed from the press and cut into small blocks of approximate dimensions 10x15x2 cm. These blocks are placed in the whey preheated between 90-92°C for 40-60 min. At the end of this step, the blocks float on the surface of the surface of the whey. Cooking cheese in whey destroys the rennet and a large part of the microbial flora and denatures the endogenous enzymes **(Kaminarides et al., 2007).**

This step of cooking in whey does not occur in the manufacture of other types of cheese, it is specific to Halloumi cheese, what gives this cheese distinct sensory and textural

characteristic and the ability to be cooked at high temperatures without melting **(Papademas and Robinson, 1998; Raphaelides et al., 2006).**

Figure 13: Photography of cheese cooking

III.2.8. Salting and enrichment

After cooking, each block is removed and allowed to cool and then sprinkled with dry salt (3%) over the entire surface m/m. The salt plays an important role in the manufacture of cheese; its main functions are taste and preservation, it also has a role in the draining. In terms of sensory characteristics, salt improves the flavor, reduces the perception of bitterness and increases the elasticity of the cheese **(Roger, 1979; Eck, 1987; Kamleh et al., 2012)**

Figure 14: Photography of the enriched cheese

III.2.9. Storage in brine

In this step the cheese blocks were stored in boxes containing 9 % salt brine for 24 hours. These blocks were then removed from the brine, drained and wrapped in plastic film and stored in a refrigerator (0 to 6°C).

Figure 15: Photography of the cheese stored in the brine

III.3 Antioxidant activity

DPPH° is a stable free radical by virtue of the delocalization of the available electron, which causes a deep purple color (**Molyneux, 2004).** It reacts with thiol groups, amines, phenols, acids, hydroaromatic compounds, etc. This property is widely recommended and used in analytical practice (**Yodanov and Christova, 1997),** when the solution of DPPH° is mixed with that of a substance that can donate a hydrogen atom or an electron, then this causes the reduced form (1,1-diphenyl-2, 4,6-trinitrophenyl hydrazine) DPPH2 , with the loss of the purple color and appearance of a residual pale yellow color, due to the presence of the picryl group. The DPPH radical has an absorbance maximum around 517 to 520 nm, which decreases when the radical is reduced. The DPPH° method is described as simple, fast and convenient, independent of sample polarity **(Marxen et al., 2007)**, the protocol is summarized in the following figure:

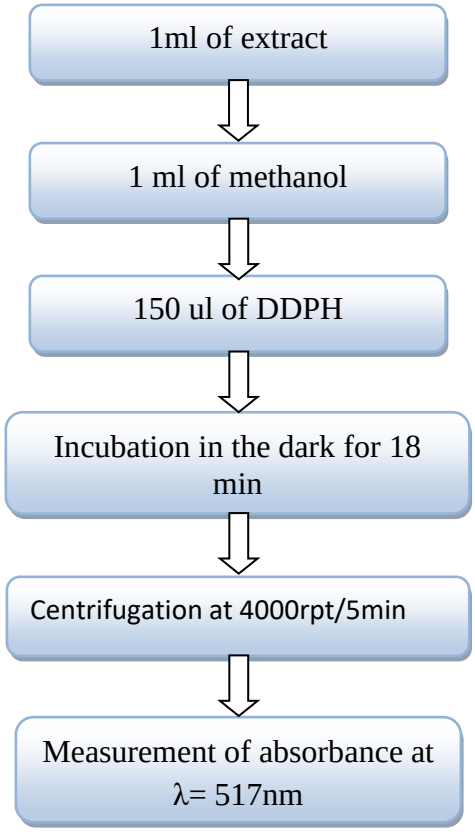

Figure 16: Steps of the DPPH° test **(Apostolidis et al., 2007)**

Scavenging capacity was measured as the decrease at 517 nm. Antioxidant activity was expressed as %DPPH-scavenging activity relative to the control, **(Masmoudi et al., 2020)** using the following equation:

% Radical scavenging activity = (AC -AS / AC) X100

Where:

AC = absorbance of the control

AS = absorbance of the sample extract

-The effect of the concentration of the different methanolic extracts on the reduction of the DPPH° radical was also tested.

.III.4. Physic-chemical analysis of Halloumi cheese

The physico-chemical analyses of cheese are made within the SARL Soummam dairy (Akbou, Bejaia). In order to determine the quality of prepared cheese, we are limited to physico-chemical analysis considered as the basic analysis:

- ✓ Moisture.
- ✓ Crude protein determinations;
- ✓ Lipid determinations;
- ✓ Carbohydrate determinations;
- ✓ Salt content;

III.4.1. Determination of Moisture

The moisture of a food is the amount of free water it contains. Its determination is done by drying in a ventilated oven at 105°C until the mass of the food remains constant.

- ☞ 3 Petri dishes containing (2g) of each cheese sample were placed in a ventilated oven at 105°C for 3h. The results of the moisture content of the cheese were expressed as a percentage using the following formula:

Where:

$$MC\% = (P0-P1/P)*100$$

H%: Moisture.
P0: Mass of Petri dish + sample before heating (g).
P1: Mass of Petri dish + sample after heating (g).
P: Mass of the test sample (g).
*Where P0: correspond to the loss in weight (g) on drying and P1: correspond to the initial weight of sample (g).

III.4.2. Crude protein determination

Unlike sugars and lipids, proteins contain nitrogen. This property will be exploited in the method for determining protein content in foods. The Kjeldahl method was used for the determination of crude protein (total nitrogen) of cheese samples. It is based on the transformation of organic nitrogen into ammonium sulfate under the action of sulfuric acid and in the presence of a catalyst **(Lecoq, 1965)**.The latter is performed in three phases: digestion (mineralization), distillation and titration **(Kjeldahl, 1883)**.

- **Mineralization:** Into a Kjeldahl matron, 0.5 g of a ground sample, 2 g of a catalyst (selenium, potassium sulfate, and copper sulfate), and 20 ml of concentrated H_2SO_4 (97%) are introduced. This mixture shows a black coloration. Then, the matras is heated until the black color turns into clear color, at which time the organic nitrogen is converted into inorganic nitrogen. After cooling, the mineralized sample is transferred to a flask whose volume is adjusted to 100 ml with distilled water.
- **Distillation:** In a matras, 20 ml of the contents of the flask, 50 ml of distilled water and 50 ml of the soda (40%) were introduced. In parallel, 20 ml of boric acid (H_3BO_3) (4%) with a few drops of colored indicators (methylene red and methylene blue) are added. The distillation stops after 4 minutes from the beginning of boiling.
- **Titration:** Since boric acid was used as a recovery solution, the excess borate anions are then titrated with sulfuric acid (0.02N) until the color changes from green to pink-purple.Total nitrogen is calculated according to the following formula:

$$\mathrm{N\%} = \frac{(V1 - V0)}{\mathrm{P\ test}} \times 100$$

Where: **N%:** Percentage of nitrogen; **P%:** Percentage of Protein. **V1:** Volume of concentrated sulfuric acid (ml); **V0 :** Volume of concentrated sulfuric acid used for the control (ml); **P test :** The mass of the test sample (g).

*The total nitrogen content is converted to crude protein content using the following formula:

Crude protein rate (%) = Total N (%) x 6.25

Where 6.25 is a conversion factor based on the average protein nitrogen content.

III.4.3. Determination of Fat

This International Standard specifies the Van Gulik method for the determination of the fat content (mass fraction) of cheeses. **(ISO 3432|FIL 221:2007).**

- Principle and procedure:

The Van Gulik method is a conventional technique which, applied to a cheese which gives a fat content (FF), expressed in grams per 100 g of cheese, equivalent to that obtained by the reference method (ISO 1735|FIL 5). It is based on the dissolution of the proteins by sulfuric acid and the separation of the fat by centrifugation in a butyrometer with a perforated

cup, this separation is favoured by the addition of the addition of iso-amyl alcohol. A test sample of 3g of cheese is weighed into a perforated glass the latter is placed in a cheese butyrometer. Then, the acid (H2SO4, d=1.52) is added until the cup emerges, the whole is put in a water bath at 70°C for 3h. Then, 1 ml of iso-amyl alcohol (3-methyl-1-butanol) is added to the sample, and then the volume is completed with sulfuric acid up to the 35% graduation. The butyrometer is centrifuged at 1000 Rpm/10 min. After centrifugation, the result is read on the graduations of the butyrometer.

- Expression of the results:

The fat content of the fine product. Expressed in g per 100g of cheese is:

$$\boldsymbol{MG}(\%) = \boldsymbol{B} + \boldsymbol{A}$$

Where:

A: the reading taken at the lower end of the MG column.
B: the reading taken at the upper end of the MG column.

III.4.4. Determination of carbohydrates

Sugars, also called carbohydrates or Cn(H2O)n carbohydrates, are compounds with numerous hydroxyl groups (-OH) responsible for their very hydrophilic character. The presence of carbonyl (-C=O), aldehyde or ketone groups gives them a reducing character **(Boukhary, 2008).** There are many methods for the determination of carbohydrates. Some of these methods use the reducing or non-reducing power of sugars.

The Lane-Eynon method is a volumetric method for determining total reducing sugars in foods. It is an empirical method that relates, using a conversion table, an amount of reducing sugars contained in a volume of food solution required to reduce a given volume of Fehling reagent. **(Edouard Tabet, 2009).**

The method is based on the ability of reducing sugars to reduce cupric hydroxide to cuprous oxide. A given volume of Fehling's reagent (10 ml or 25 ml) is titrated under heat with a solution of the food containing the reducing sugar(s). The Methylene Blue indicator is used to make the blue color of the Fehling reagent disappear (turning point). The volume of food solution used for the titration is converted into mg of reducing sugars using a conversion table. **(Edouard Tabet, 2009).**

Procedure:

- Accurately weigh into a beaker 12.5g of cheese then dilute the sample with distilled water to 250ml.
- Prepare a mixture of mixed Fehling solution (12.5ml Fehling A + 12.5ml Fehling B) in a 250ml conical flask and mix well.
- Fill a 50ml burette with the sample solution.
- Add about 13.5ml of cheese solution to the mixed Fehling solution from the burette, then heat on a Bunsen burner.
- Boil for two minutes and add 03 drops of the methylene blue indicator.
- Add without interrupting the boiling 2-3 drops of dextrose solution until the blue color disappears completely.

III.4.5. Salt content

To date, there is no method to directly measure the amount of salt (NaCl) in dairy products, it is necessary to use an indirect approach (by calculation). Two ways are possible: the chloride way and the sodium way **(Anonymous, 1).**

The "chloride" method is mainly used in the dairy sector. The principle is to measure the Cl- ions and to estimate the "salt" content by calculation, assuming that all chlorides come from NaCl. The method is standardized internationally by **ISO 5943:2006.**

Principle:

The general principle is a silver nitrate titration of Cl- ions, of a suspension of "acidified cheese" using a specific electrode.

III.5. Microbiological analysis

The microbiological analyses were carried out according to the protocols of the executive decree of 27/05/1998 of the official newspaper of the Algerian Republic N° 35. This last aims on the one hand to preserve the organoleptic and sensory characteristics of the product, thus to lengthen its lifespan and on the other hand to prevent the cases of food poisoning related to the presence of pathogenic microorganisms before the transmission to the consumer.

- **Preparation of the stock solution and decimal dilutions:**

Under aseptic conditions, 10 g of cheese are homogenized in 90 ml of physiological water, which forms the stock solution (10-1). A series of decimal dilutions is performed by taking 1 ml of the stock solution in 9 ml of physiological water, which constitutes the dilution (10-2), after homogenization of the latter; the same operation is repeated to obtain successive dilutions to prepare the number of decimal dilutions appropriate for the enumeration of each flora.**(J.O.R.A, 2004).**

Table VIII. Microbiological analysis of cheeses

Germs sought	Culture media	Temperatures and incubation time	References
Staphylococcus aureus	Baird Parker agar	37°C /48h	**(J.O.R.A, 2014)**
total coliforms	VRBL	30°C/24h	**(J.O.R.A, 2017)**
fecal coliforms	VRBL	44°C/24h	**(J.O.R.A, 2017)**
Yeasts and molds	YGC	22°C/5D	**(N.A.21 10)**
Aerobic germs	PCA	30°C/3D	**(J.O.R.A, 2017)**

VRBL: Violet Red Bile Agar
OGA: Yeasts extract glucose chloramphenicol agar
PCA: Plant Count Agar

- **Salmonella detection:**

A pre-enrichment in Ringer¼ liquid is performed by 25g of cheese in 225 mL of the medium and incubated at 37°C/ 24 h, followed by an enrichment followed by enrichment by spiking a few drops of the pre-enrichment medium into 9 mL of sterile cysteine selenite broth and incubation at 37°C /24 h. Finally, streak isolation is performed on bismuth sulfite medium with incubation at 37°C/24 h **(N.A.26 88).**

Results and Discussion

IV.1. Analysis of variance and design validation

This analysis makes it possible to test the relevance of the variables involved in the model studied and to graphically represent the importance of each factor on the response studied, in this case the yield. Regression coefficients for intercept, linear, quadratic and interaction terms were calculated as represented in **table IX**.

Table IX. The ANOVA analysis for the experimental results of yield

Source	**DF**	**Sum of squares**	**Mean squares**	**Rapport F**	**P-Value**
Model	9	694,31652	77,1463		<0,0001*
Error	140	216,01442	1,5430		
C. Total	149	910,33093			
Lack of fit	3	201,54775	67,1826	636,2222	<0,0001*
Pure error	137	14,46667	0,1056		
Total error	140	216,01442			
Cow milk(400,800)=X1	1	76,05000	76,05000	49,2884	<0,0001*
Goat milk(200,600)=X2	1	0,90312	0,90312	0,5853	0,4455
Milk powder(40,80)=X3	1	255,97012	255,97012	165,8955	<0,0001*
X_1*X_2	1	4,69225	4,69225	3,0411	0,0834
X_1*X_3	1	100,17225	100,17225	64,9221	<0,0001*
X_2*X_3	1	15,37600	15,37600	9,9653	0,0020*
X_1^2	1	21,84433	21,84433	14,1574	0,0002*
X_2^2	1	76,91856	76,91856	49,8513	<0,0001*
X_3^2	1	127,28164	127,28164	82,4919	<0,0001*

R^2=76 %, **R^2 Adj**= 0, 74, **CV=** (Coefficient of variation RMSE / MEAN OF REPONSE) *100 **CV**= 7, 20 %

As shown in the table above, two factors (Cow's milk and Milk powder) have their p-values below 0.05, which means that they are significantly different from zero at the 95% confidence level. R^2, being an indicator value of the degree of explanation of the influence of the factors on the response, is equal to 76%, which leads to say that the model could be well applied to predict the influence of the studied factors on the cheese enrichment. In addition, the Lack of-fit value (**Tab.IX**) was designed to determine whether the chosen model is valid to describe the observed data **(Achat et al., 2012),** so that this value is greater than 0.05 which is witness to the validity of this design.

The cow's milk, goat milk and powder milk concentration significantly influence the yield of cheese. Indeed as shown in the table above, seven factors (X_1, X_3, X_1*X_3, X_2*X_3, X_1^2

X_2^2 and X_3^2) give a p-value less than of **0.05**. The predicted models are expressed by second-order polynomial equations 1:

$$\mathbf{Y= 17,83 – 0,97X_1 + 1,78X_3 + 1,58X_1X_3+ 0,62X_2X_3- 0,76\ X_1^2+1,44\ X_2^2-1,85X_3^2}\text{……..(1)}$$

The equation 1 demonstrate, that the factor X3 and the interaction between the two factors X_1X_2, X_2X_3 and X_2^2 acts positively on the yield of the cheese, and the factors X_1, X_1^2, X_2^2 and X_3^2 acts negatively on the yield of the cheese.

IV.2. Antioxidant activity

The DPPH radical is usually used as a substrate to evaluate the antioxidative action of antioxidants by determining the free radical-scavenging ability of various samples **(Achat et al., 2012**).

Antioxidant activity expresses the capacity to reduce free radicals. These free radicals are criticized in various pathologies. In order to compensate for the endogenous defense system, research is oriented in the discovery of antioxidant molecules. Thus to evaluate the antioxidant activity of our extracts, we used the DPPH method. This free radical presents a very dark coloration. When it is trapped by antioxidant substances, the reduced form gives the solution a pale yellow color. The shift to this coloring and the intensity of the intensity of the coloration of the free form in solution depends on the nature, the concentration and strength of the anti-free radical substance.

The antioxidant activity of the four sample cheese towards the DPPH radical was evaluated by spectrophotometry by following the reduction of this radical which accompanied by its color change from violet to yellow, at 517nm. The antioxidant activity of ascorbic acid was evaluated as standard.

The findings of the antiradical activity are expressed in percentages. **Figures 17** shows percentage of inhibition of different extract of cheese (control cheese, enriched with essential oil of rosemary, with powder and with the leaves respectively).

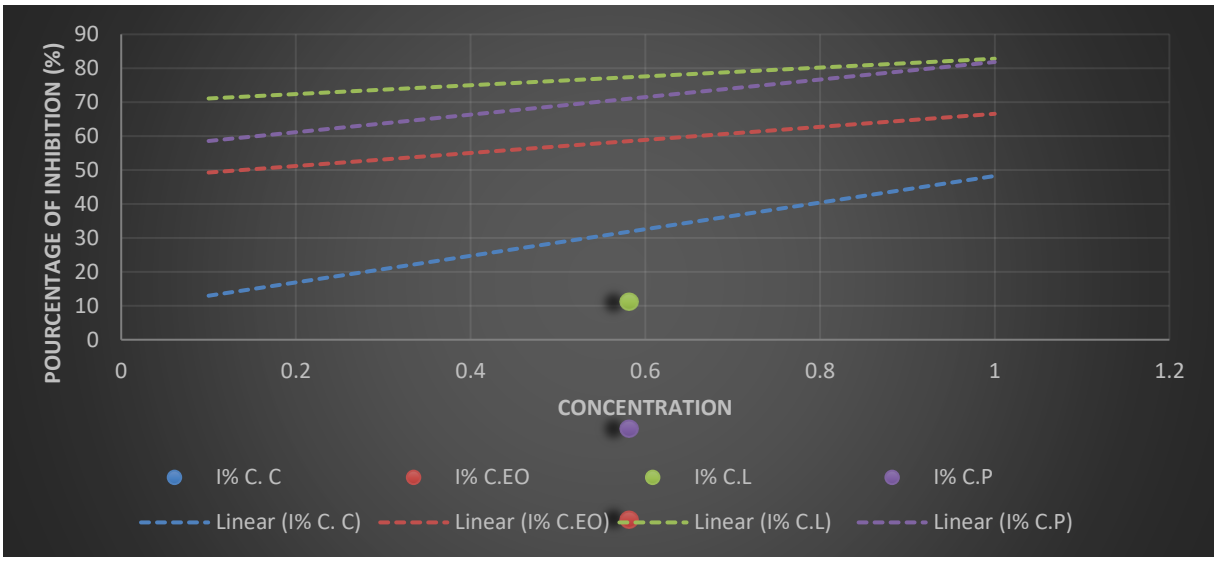

Figure 17: Antiradical activity (DPPH°) of produced cheeses.

The antioxidant activity of the control cheese is between 11% and 47% and increases with increasing concentration.

This figure showed that cheeses enriched with rosemary leaves as well as rosemary powder represent higher percentages of inhibition (81.51%) than cheese enriched with rosemary essential oil.

The results of this test can be expressed in terms of IC_{50}, which is defined as the concentration of the extract necessary to inhibit 50% of the DPPH° radical **(Apostolidis et al., 2007)**. A low IC_{50} corresponds to a high reducing capacity, the more antioxidants are added, the more DPPH° radicals are reduced.

According to the results obtained in figure 18, a better scavenger activity to the DPPH° radical was attributed to the extracts of enriched cheeses with IC_{50}: 10.31 mg/ml, 0.579 mg/ml and 0.736 mg/ml for C.C, C.EO and C.P respectively. However, the cheese with dried leaves presents a higher antioxidant power with an IC_{50} of 0.499 mg/ml.

IV.3. Results of the analysis of Halloumi cheese

IV.3.1 Physic-chemical analysis

The results of the determination of fat, carbohydrates, proteins and chlorides are presented in the **table X**.

Table X. Results of some physic-chemical parameters of Halloumi cheese.

Parameter	C. C	C. EO	C. P	C. L.
Moisture	68,40	64,89	65,53	66,98
EST	31,60	35,11	34,47	33,02
Proteins	38	39	38	38
Fat	13,1	14,50	15,32	13,70
Carbohydrates	0,86	0,87	0,85	0,80
Salt	5,27	4,40	4,85	4,40

C.C: control cheese;
C. EO: cheese enriched with essential oil;
C. P: cheese enriched with rosemary powder;
C. L: cheese enriched with rosemary leaves.

According to the results obtained, the values of fat and protein of the different cheeses are very close, except for the C. EO cheese, which has a higher value than the others. This is explained by the fact that the latter is enriched with essential oil. The fat and protein contents of goat milk are higher than those of cow milk according to **Carole and Vignola (2002).**

For carbohydrates, the results obtained show that the 4 cheese samples have almost the same content. As cow's milk and goat's milk have similar carbohydrate contents (4.7% and 4.4% respectively) according to **Carole and Vignola (2002).**

For chlorides, the contents are very close, except for the C.C. cheese which has a slightly higher salt content, this may be due to the fact that it is the first cheese made, the time left in the salt brine is higher than the other samples.

The results of the physico-chemical analysis of the cheeses show that they are a good source of protein (38-39%), quite rich in fat (13-15%), not rich in sugar (0.8%). On the other hand, the salt content is quite high (4.4 to 5.2%).

IV.3.2. Microbiological analysis

The objective of microbiological analysis, when a product is intended for consumption, is to guarantee a certain hygienic safety and organoleptic quality level and to preserve the health of the consumer.

The results of the microbiological analysis of the cheeses are expressed in CFU/ml and are shown in the **table XI**.

Table XI. Results or microbiological analyses of different cheeses

	Total coliforms/g	**Fecal coliforms/g**	**Aerobic germs/g**	***Staphylococcus aureus*/g**	**Yeasts and molds/g**	**Salmonella /25g**
C. C	$1{,}1.10^2$	69	Absent	Absent	Absent	Absent
C. EO	$1{,}2.10^2$	67	Absent	Absent	Absent	Absent
C. P	10^2	73	Absent	Absent	Absent	Absent
C. L	10^2	65	Absent	Absent	Absent	Absent

All the results of microbiological analysis obtained revealed that the 4 samples of cheese have a satisfactory microbiological quality, despite the fact that the samples were analyzed after almost a month from the date of manufacture. This is due to the fact that Halloumi cheese is stored in brine with 9% salt which allows its conservation. Adding also to the good hygienic conditions during the preparation of these cheeses.

Conclusion

During this study we were able to learn and master the manufacturing process of Halloumi cheese which is not known in the Mediterranean region. It is part of the half-hard cheeses with cooked paste. It is prepared preferably with a mixture of goat's milk, sheep's milk and cow's milk. For the formation of the curd, only rennet is used only rennet is used, no lactic ferments.

Four types of Halloumi cheese were prepared: control cheese made from cow's milk and goat's milk, **C. EO, C. P** and **C. L** which have the same composition as the control cheese enriched with rosemary essential oil, rosemary powder and finally rosemary leaves respectively.

The results of the physic-chemical analyses of the cheeses elaborated show that they are a good source of proteins **(38 to 39%)**, quite rich in fat **(13 to 15%)**, are not rich in sugar **(0.8%)**. On the other hand, the salt content is quite high **(4.4 to 5.2%).**

The antioxidant capacity of the different cheese enrichments obtained from *Rosmarinus officinalis* was evaluated by the DPPH° free radical scavenging method. In this work, dried rosemary leaves have demonstrated to be the most effective in preventing the oxidation (81.51%), with an IC_{50} of 0.499 mg/ml followed by rosemary powder (IC_{50}: 0.579 mg/ml) and essential oil (IC_{50}: 0.736 mg/ml), finally the lowest percentage was attributed to cheese without enrichment (IC_{50}: 10.31 mg/ml).

The results of microbiological analyses show that these cheeses have a good microbiological quality, which makes them suitable for consumption.

In the future, certain points remain to be studied in greater depth, and it would therefore be interesting to complete this study by:

- ✓ The phytochemical analysis (total polyphenols and flavonoids) of the rosemary and the finished products;
- ✓ The other physic-chemical analysis such as calcium and vitamins;
- ✓ The stability of the prepared cheeses during the shelf life;
- ✓ The application in industrial scale application to bring this new product on the Algerian market.

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Appendix

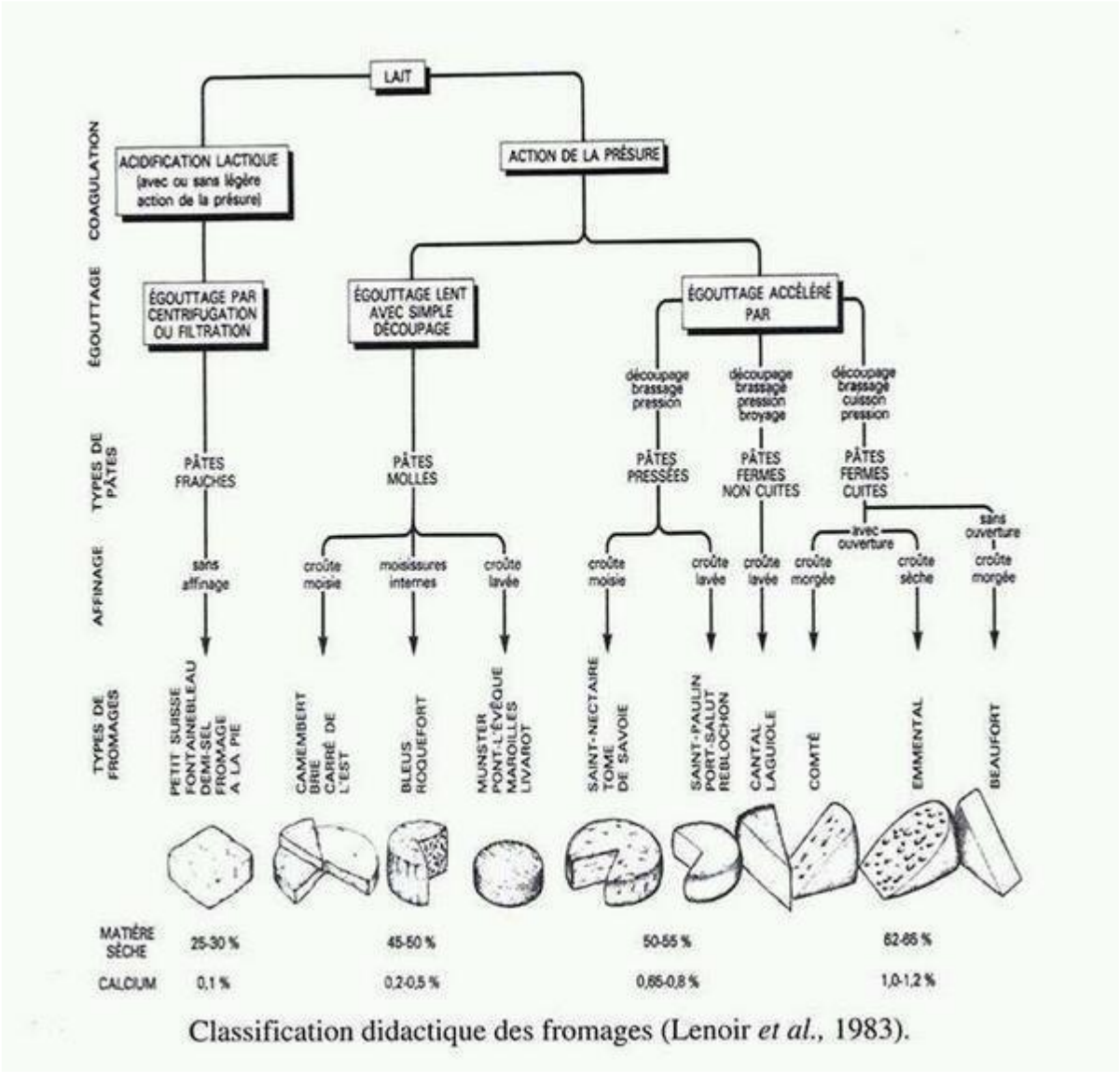
Appendix 1

Classification of cheese according to consistency, fat content and main maturing characteristics **A-6(FAO/OMS, 1994)**

Formula I		**Formula II**		**Formula III**
TEFD(%)	The first element of the the name will be :	**MGES(%)**	The second element of the name will be :	According to the main characteristics of refining
<51	Exra hard dough	>60	Extra fat	1 : refined in yeast A : mainly on the surface B : Mainly in Mass 2 : Mould-ripened A : mainly on the surface B : Mainly in mass
		45-60	Full fat	
49-56	Hard dough			
		25-45	Medium fat	
54-63	Semi-hard dough			
		10-25	Four-fat	
61-69	Semi-soft dough	<10	Lean	
>67	Soft dought			

Appendix 2

Didactic classification of cheeses according to Lenoir et al 1983.



Classification didactique des fromages (Lenoir *et al.*, 1983).

Appendix 3

Questionnaire d'Analyse Sensorielle du Fromage Halloumi (part1)

Nom et prénom :……..
Date :……..

Sexe :……..... **Age :…….**
Poste :…….

Cinq échantillons de fromage Halloumi non cuits vous sont présentés, il vous est demandé d'évaluer différentes caractéristiques et attribuer une note pour chaque échantillon selon votre appréciation.

NB : Veuillez rincer la bouche après chaque dégustation d'échantillon.

1. **Couleur :**

1. Pas intense 2.Peu intense 3. Moyennement intense 4. Fortement intense

5. Très fortement intense.

Echantillon 1	Echantillon 2	Echantillon 3	Echantillon 4	Echantillon 5

2. **Odeur : (sans gouter le fromage)**

1. Absente 2. Faible 3. Moyenne 4. Forte 5. Très forte

Echantillon 1	Echantillon 2	Echantillon 3	Echantillon 4	Echantillon 5

3. **Salinité :**

1. Absente 2.Faible 3.Moyenne 4.Forte 5.Très forte

Echantillon 1	Echantillon 2	Echantillon 3	Echantillon 4	Echantillon 5

4. **Intensité de l'arôme** :(après avoir gouté le fromage)

1. Absente 2.Faible 3.Moyenne 4.Forte 5.Très forte

Echantillon 1	Echantillon 2	Echantillon 3	Echantillon 4	Echantillon 5

5. **Tendreté :**

1. Absente 2.Faible 3.moyenne 4.Tendre 5.Trés tendre

Echantillon 1	Echantillon 2	Echantillon 3	Echantillon 4	Echantillon 5

6. Dureté :

1. Absente 2.Faible 3.Moyenne 4.Forte 5.Très forte

Echantillon 1	**Echantillon 2**	**Echantillon 3**	**Echantillon 4**	**Echantillon 5**

7. Elasticité :

1. Absente 2.Faible Moyenne 4.Forte 5.Très forte

Echantillon 1	**Echantillon 2**	**Echantillon 3**	**Echantillon 4**	**Echantillon 5**

8. Texture en bouche :

1. Fondante 2.Granuleuse 3.Lisse 4. Collante 5.Friable

Echantillon 1	**Echantillon 2**	**Echantillon 3**	**Echantillon 4**	**Echantillon 5**

9.Préférence :

Attribuer une note de 1 à 9 pour chaque échantillon selon votre préférence, sachant que 1 correspond au moins préféré et 9 au plus préféré, comme présenté dans l'échelle ci-dessous :

1 : Extrêmement désagréable
2 : Très désagréable
3 : Assez désagréable
4 : Désagréable
5 : Ni agréable ni désagréable
6 : Assez Agréable
7 : Agréable
8 : Très agréable
9 : Extrêmement agréable

Echantillon 1	**Echantillon 2**	**Echantillon 3**	**Echantillon 4**	**Echantillon 5**

« Merci pour votre participation »

Appendix 4

Questionnaire d'Analyse Sensorielle du Fromage Halloumi (part2)

Nom et prénom :……..
Date :……..

Sexe :……..... **Age :…….**
Poste :…….

Cinq échantillons de fromage Halloumi non cuits vous sont présentés, il vous est demandé d'évaluer différentes caractéristiques et attribuer une note pour chaque échantillon selon votre appréciation.

NB : Veuillez rincer la bouche après chaque dégustation d'échantillon.

1. **Couleur :**

1. Pas intense 2.Peu intense 3. Moyennement intense 4. Fortement intense

5. Très fortement intense.

Echantillon 6	**Echantillon 7**	**Echantillon 8**	**Echantillon 9**	**Echantillon 10**

2. **Odeur : (sans gouter le fromage)**

1. Absente 2. Faible 3. Moyenne 4. Forte 5. Très forte

Echantillon 6	**Echantillon 7**	**Echantillon 8**	**Echantillon 9**	**Echantillon 10**

3. **Salinité :**

1. Absente 2.Faible 3.Moyenne 4.Forte 5.Très forte

Echantillon 6	**Echantillon 7**	**Echantillon 8**	**Echantillon 9**	**Echantillon 10**

4. **Intensité de l'arôme** :(après avoir gouté le fromage)

1. Absente 2.Faible 3.Moyenne 4.Forte 5.Très forte

Echantillon 6	**Echantillon 7**	**Echantillon 8**	**Echantillon 9**	**Echantillon 10**

5. **Tendreté :**

1. Absente 2.Faible 3.moyenne 4.Tendre 5.Trés tendre

Echantillon 6	**Echantillon 7**	**Echantillon 8**	**Echantillon 9**	**Echantillon 10**

6. **Dureté :**

1. Absente 2.Faible 3.Moyenne 4.Forte 5.Très forte

Echantillon 6	Echantillon 7	Echantillon 8	Echantillon 9	Echantillon 10

7. Elasticité :

1. Absente 2.Faible Moyenne 4.Forte 5.Très forte

Echantillon 6	Echantillon 7	Echantillon 8	Echantillon 9	Echantillon 10

8. Texture en bouche :

1. Fondante 2.Granuleuse 3.Lisse 4. Collante 5.Friable

Echantillon 6	Echantillon 7	Echantillon 8	Echantillon 9	Echantillon 10

9.**Préférence** :

Attribuer une note de 1 à 9 pour chaque échantillon selon votre préférence, sachant que 1 correspond au moins préféré et 9 au plus préféré, comme présenté dans l'échelle ci-dessous :

1 : Extrêmement désagréable
2 : Très désagréable
3 : Assez désagréable
4 : Désagréable
5 : Ni agréable ni désagréable
6 : Assez Agréable
7 : Agréable
8 : Très agréable
9 : Extrêmement agréable

Echantillon 6	Echantillon 7	Echantillon 8	Echantillon 9	Echantillon 10

« Merci pour votre participation »

Appendix 5

Questionnaire d'Analyse Sensorielle du Fromage Halloumi (part3)

Nom et prénom :……..
Date :……..

Sexe :……..... **Age :…….**
Poste :…….

Cinq échantillons de fromage Halloumi non cuits vous sont présentés, il vous est demandé d'évaluer différentes caractéristiques et attribuer une note pour chaque échantillon selon votre appréciation.

NB : Veuillez rincer la bouche après chaque dégustation d'échantillon.

1. **Couleur :**

1. Pas intense 2.Peu intense 3. Moyennement intense 4. Fortement intense

5. Très fortement intense.

Echantillon 11	**Echantillon 12**	**Echantillon 13**	**Echantillon 14**	**Echantillon 15**

2. **Odeur : (sans gouter le fromage)**

1. Absente 2. Faible 3. Moyenne 4. Forte 5. Très forte

Echantillon 11	**Echantillon 12**	**Echantillon 13**	**Echantillon 14**	**Echantillon 15**

3. **Salinité :**

1. Absente 2.Faible 3.Moyenne 4.Forte 5.Très forte

Echantillon 11	**Echantillon 12**	**Echantillon 13**	**Echantillon 14**	**Echantillon 15**

4. **Intensité de l'arôme** :(après avoir gouté le fromage)

1. Absente 2.Faible 3.Moyenne 4.Forte 5.Très forte

Echantillon 11	**Echantillon 12**	**Echantillon 13**	**Echantillon 14**	**Echantillon 15**

5. **Tendreté :**

1. Absente 2.Faible 3.moyenne 4.Tendre 5.Trés tendre

Echantillon 11	**Echantillon 12**	**Echantillon 13**	**Echantillon 14**	**Echantillon 15**

6. **Dureté :**

1. Absente 2.Faible 3.Moyenne 4.Forte 5.Très forte

Echantillon 11	Echantillon 12	Echantillon 13	Echantillon 14	Echantillon 15

7. Elasticité :

1. Absente 2.Faible Moyenne 4.Forte 5.Très forte

Echantillon 11	Echantillon 12	Echantillon 13	Echantillon 14	Echantillon 15

8. Texture en bouche :

1. Fondante 2.Granuleuse 3.Lisse 4. Collante 5.Friable

Echantillon 11	Echantillon 12	Echantillon 13	Echantillon 14	Echantillon 15

9. Préférence :

Attribuer une note de 1 à 9 pour chaque échantillon selon votre préférence, sachant que 1 correspond au moins préféré et 9 au plus préféré, comme présenté dans l'échelle ci-dessous :

1 : Extrêmement désagréable
2 : Très désagréable
3 : Assez désagréable
4 : Désagréable
5 : Ni agréable ni désagréable
6 : Assez Agréable
7 : Agréable
8 : Très agréable
9 : Extrêmement agréable

Echantillon 11	Echantillon 12	Echantillon 13	Echantillon 14	Echantillon 15

« Merci pour votre participation »

Abstract:

The present work has been undertaken firstly with the aim of elaborating a Halloumi cheese, a semi-hard cheese with cooked paste, its specific characteristic is that it does not melt during cooking. It is the traditional cheese of Cyprus, it is also made in Lebanon, Series and Turkey but is not known at in the Mediterranean region.Thus, the aim of this study is the enrichmentHalloumi cheese with plant rosemary (*Rosmarinus officinalis*)for the improvement of its nutritional and organoleptic value, since rosemary is rich in bioactive substances (phenolic compounds and essential oil).Four types of Halloumi cheese have been prepared: **C. Cheese**(60% goat milk, 40% cow milk and milk powder), **Cheese. P** (C. Cheese enriched with rosemary powder), **Cheese. L** (C. Cheese enriched with rosemary leaves) and finally **Cheese. EO** (C. Cheese enriched with rosemary essential oil).The results of the physic-chemical analysis of the elaborated cheeses show that they are a good source of proteins (35 to 39%), quite rich in fat (13 to 15%), are not rich in sugar (0,8%). On the other hand, the salt content is quite high (4.4 to 5.2%). The results of microbiological analyses show that these cheeses have a good microbiological quality.The antioxidant activity was carried out on the four types of cheese, the results obtained confirm that the addition of rosemary powder and leaves provide to the cheeses an important antioxidant activity. The data obtained after enrichment according to the experimental design indicate that the best percentage of goat milk is 60% with an optimal percentage of cow milk of 40%, as well as to the improvement of the organoleptic characteristics of the cheese essentially the aroma.

Keywords: Halloumi cheese, rosemary, experimental design, antioxidant activity.

Résumé :

Le présent travail a été entrepris tout d'abord dans le but d'élaborer un fromage Halloumi, un fromage à pâte mi-dure et cuite, sa caractéristique spécifique est qu'il ne fond pas pendant la cuisson. C'est le fromage traditionnel de Chypre, il est également fabriqué au Liban, en série et en Turquie mais n'est pas connu dans la région méditerranéenne. Ainsi, le but de cette étude est l'enrichissement du fromage Halloumi avec du romarin végétal (Rosmarinus officinalis) pour l'amélioration de sa valeur nutritionnelle et organoleptique, puisque le romarin est riche en substances bioactives (composés phénoliques et huile essentielle).Quatre types de fromage Halloumi ont été préparés : **Fromage C.** (60% lait de chèvre, 40% lait de vache et poudre de lait), **Fromage. P** (Fromage C. enrichi en poudre de romarin), **Fromage. L** (fromage C. enrichi en feuilles de romarin) et enfin le **Fromaged EO** (C. Fromage enrichi à l'huile essentielle de romarin). Les résultats de l'analyse physico-chimique des fromages élaborés montrent qu'ils sont une bonne source de protéines (35 à 39%), assez riches en matières grasses (13 à 15%), ne sont pas riches en sucre (0,8%). Par contre, la teneur en sel est assez élevée (4,4 à 5,2%). Les résultats des analyses microbiologiques montrent que ces fromages ont une bonne qualité microbiologique. L'activité antioxydante a été réalisée sur les quatre types de fromage, les résultats obtenus confirment que l'ajout de poudre et de feuilles de romarin apportent aux fromages une activité antioxydante importante. Les données obtenues après enrichissement selon le plan expérimental indiquent que le meilleur pourcentage de lait de chèvre est de 60% avec un pourcentage optimal de lait de vache de 40%, ainsi qu'à l'amélioration des caractéristiques organoleptiques du fromage essentiellement l'arôme.

Mots clés : Fromage Halloumi, romarin, plan d'expérience, activité antioxydante.