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Réf : .....

Mémoire de Fin de Cycle

En vue de l'obtention du diplôme

MASTER

Thème

Elaboration d'un aliment à valeur ajoutée : suivi micobiologique et physicochimique à Cevital

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DEDICATION

I dedicate this work,

To my parents; to my father the extraordinary man who endowed me with a dignified education, his support made me what I am today. To the one who overwhelmed me with tenderness and hope, my dear mother, I cannot thank you enough for your kindness, for your sacrfices, for the woman you have made me today.

To my dear sister Melaaz, her husband and childrens who have given me a lot of love and encouragement.

To my brother Ziri, his wife Samira and their daughter for their advices and love.

To my brother Lyes for his presence at my side despite the distance that separates us.

To Mr. Khaled. N for his help during our work.

To my cousins Walid, Mohand and Milida for their presence.

To my friends Thimouzgha and Athman for being at my side.

To my precious partner and friend Sarah and her family for their understanding and sympathy.

Thinhinan

DEDICATION

I dedicate this work

To my dear parents, thanks for your faith in me, may God give you health and happiness.

To My dearest sisters Salima and Nesrine, my brother mohamed who have always been my strength to face the different obstacles of life.

To my wonderful nephews Fares, Kiyan and my lovely niece Cerine.

To my friends Redha, Cici, Hocine, and Tina who have never stopped supporting me.

To my binome and dear friend Thinhinan and her family with whom i shared unforgettable moments.

Sarah

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Summary

List of abbreviations:

°C: Celcus degrees

CFU: Colony-Forming Unit.

DPPH: 2, 2-Diphenyl-picrylhydrazyl

EO: Essential Oil

G: Gram

KHz: Kilohertz

MAE: Microwave assisted extraction

MHz : Megahertz

MW: Microwave

Nm: Nanometer

pH: Hydrogen Potential

ppm: parts per million

REO: Rosemary essential oil

rpm: Revolutions per minute

US/MAHD: Ultrasound assisted microwave hydrodistillation

W: Watt

µl: Micro liter

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Introduction

Nowadays, consumers are concerned about the negative effect of synthetic chemicals in food and have a huge interest in the consumption of food without synthetic additives and rather with the use of natural preservatives.

The studies that have been carried out so far show that the extracts of medicinal plants can be incorporated in foods. The evaluation of the antimicrobial and the antioxidant properties of the extracts remain very important to exploit them in food industry as natural preservatives. **(Guesmi and Bodarousse, 2006)**.

Adding antioxidant in industrial food formulations is one among the foremost effective means to retard fat oxidation. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are frequently used in many foods. However, their use has been questioned due to issues regarding toxicity and carcinogenicity. Therefore, a big interest has been assigned to the appliance of natural antioxidants in foods, because of their potential nutritional and therapeutic effects. **(Achat et al., 2012).**

Due to their strong antioxidant and antimicrobial properties, the plants of the family *Lamiaceae*, to which rosemary belongs, is one of the most used botanical families worldwide **(Bouhdid et al., 2006).** In Algeria, this family is considered as one of the most important of the local flora from the point of view of its diversity and its representation. The antioxidant property of rosemary extract has assigned to the presence of bioactive substances; phenolic compound and essential oil which break free radical chain reactions by hydrogen atom donation and chelating metal ions **(Alizadeh et al., 2015).**

The gradual industrialization of traditional recipes has led to formulation adjustments to optimize processes, reduce costs and increase product lifetimes **(Chatterjee and Bhattacharjee, 2014).** Among these formulations, mayonnaise is an emulsion of oil in stable water composed of up to 80% oil. It is one of the most important savory dressings. It has become very consumable with the spread of fast food restaurants and often prepared at the kitchen level.

The objective of this study is to assess some quality criteria of the traditional mayonnaise enriched with the rosemary plant (phenolic extract, essential oil, rosemary dry leaves and dry leaves powder). In this context, several tests were carried out, such as physicochemical and microbiological tests followed by determination of the antioxidant capacities (DPPH° test) of different samples of mayonnaise.

In order to better situate the context of this research, a bibliography was presented on rosemary (chemical composition, biological activities and extraction methods) and mayonnaise (composition and enrichment with antioxidants).

Bibliography

Overview of rosemary

Rosmarinus officinalis. L, commonly known as rosemary, belonging to the *Lamiaceae* family, is an aromatic plant that grows wild in the Mediterranean basin and has been cultivated in many other regions. Rosemary herbs have been widely used in the traditional medicine, and cosmetics. They are also used as a natural food preservative and flavoring agent **(Hamidpour et al., 2017).**

-Vernacular names

English: Rosemary; French: Romarin; Arabic: Eklil El Djabel; Kabyle: Amzir or Aklil (**Goetz and Ghedira 2012).**

I.1 Morphological description

Rosemary is an evergreen-branched bushy shrub, attaining a height of about one meter with upright stem. The leaves are small, non-petiolate and needlelike in shape, they are dark green and shiny above, whereas it is white below **(Fig01)**. The small flowers appear in groups of two or more at the upper ends of the plant, they can be blue, purple or white. **(AI-Sereiti et al., 1999).**

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

Tab I : Taxonomic classification of rosemary **(Andrade et al., 2018)**

Figure 1 : *Rosmarinus officinalis. L*

I.2 Composition of rosemary

Rosemary has an important reservoir of potential compounds; it represents a good source of vitamins and minerals including calcium, iron, magnesium, and potassium.

As with most leafy greens, rosemary is a low-calorie, low fat food **(Tab II). (Orhan et al., 2008; Švarc-Gajić et al., 2013).**

The rosemary leaves are also quite rich in phytoconstituents namely polyphenols and essential oils. **(Rollinger, 2004).**

Table II: Chemical properties and mineral contents of rosemary**. (Orhan et al., 2008; Švarc-Gajić et al., 2013)**

Fraction	Content (100g)	Element	Content (mg\kg)
Total lipids (g)	67.7	Calcium	7792
Sugar (g)	20.7	Magnesium	1635
Fiber (g)	14.1	Phosphorus	1475
Vitamin A (I.U)	2924	Iron	330
Vitamin C (mg)	21.8	Sodium	2712
Riboflavin (mg)	0.152	Potassium	14916

I.2.1 Essentials oils

Essential oils (EOs) 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 **(Ríos, 2016).** EOs can vary according to temperature, soil conditions, altitude, the country of origin and the part of the plant **(Mariod, 2016)**

Essential oils 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).**

- **Terpene hydrocarbons:**

Terpenes are the most common class of chemical compounds found in essential oils **(Fig02);** they are synthesized in the cytoplasm of plant cells, through the mevalonic acid pathway. Terpenes have been regarded as polymers of isoprene (C_5H_8) **(Morsy, 2016).** The main terpenes are the monoter-penes (C10) and sesquiterpenes (C15), but hemiterpenes (C5), diterpenes (C20), triterpenes (C30) and tetraterpenes(C40) also exist. **(Bakkali et al., 2008).**

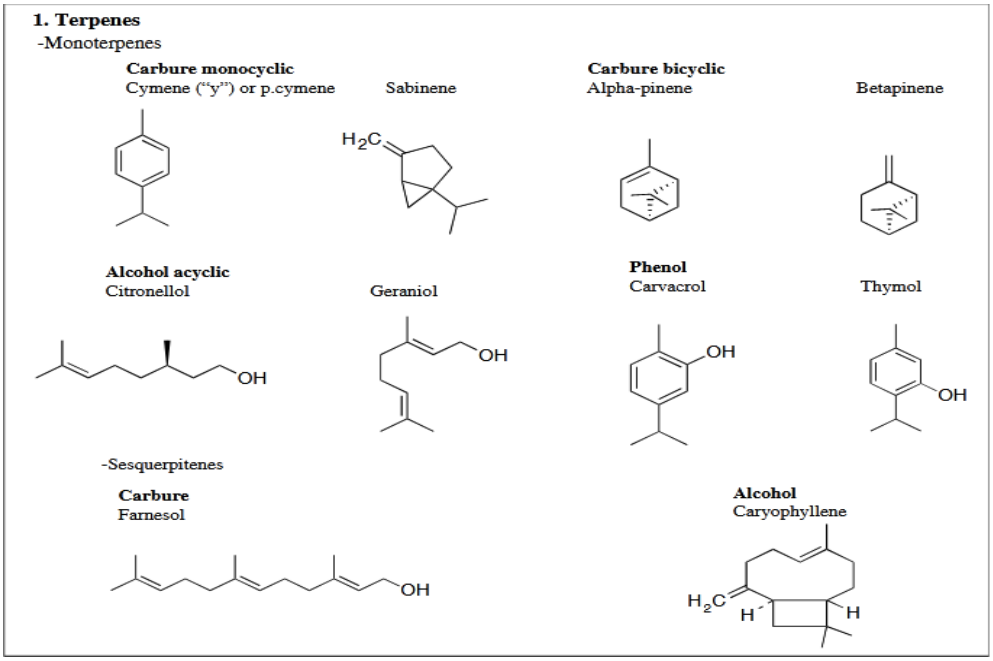


Figure 2: Chemical structures of terpene essential oils **(Bakkali et al., 2008)**

❖ Aromatic compound:

They are non-terpenic compounds **(Fig03)** biogenerated by the phenylpropanoids pathway, these compounds are usually found less than the terpenes. This molecule gives EOs a flavor and odor (**Fokou et al., 2020**).

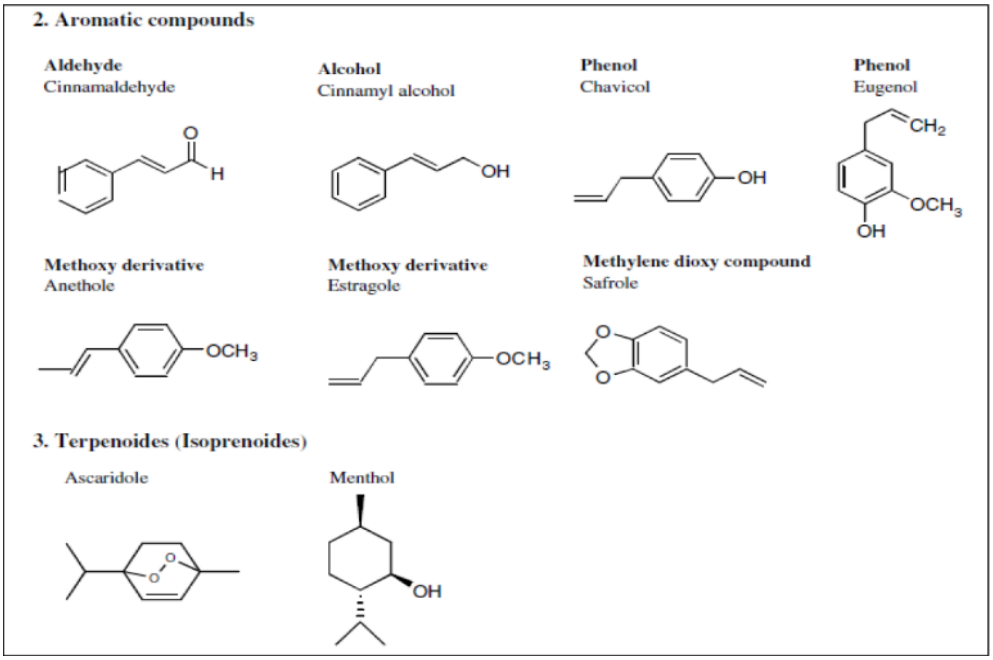


Figure 3: Chemical structures of aromatic components of essential oils **(Bakkali et al., 2008)**

Rosemary essential oil (REO) is a colorless or pale-yellow liquid, with characteristic odor of the plant. The major constituent of the REO varies from region to region. It contains α-pinene (7 to 80%), verbenone (1 to37%), camphor (1 to35%), borneol (4 to 19%), about to 10% of bronyl acetate and also camphene **(Jiang et al., 2011; Djabi and Khobizi, 2018).**

I.2.2 Phenolic composition:

Phenolic compounds are the most abundant secondary metabolites found in plants, usually related to defense responses in the plant; they contain benzene rings with one or more hydroxyl substituent, in addition to other constituents **(Lin et al., 2016)**

A large number of polyphenolic compounds have been identified in *Rosmarinus Officinalis* mainly, phenolic acids, tannins and several flavonoids. **(Fernández-Ochoa et al., 2017).**

- **Phenolic acids:**

Phenolic acids **(Fig04)** identified in the rosemary extract, are mainly hydroxybenzoic acids (C1-C6) and hydroxycinnamic acids (C3-C6) **(Mena et al., 2016).**

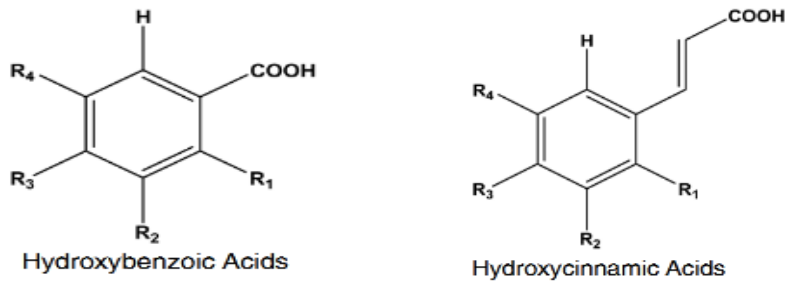


Figure 4: Structures of the phenolic acids **(Stalikas, 2007)**

- The main phenolic acids of rosemary are rosmarinic acid, caffeic acid vanillic acid, gallic acid and p-coumaric acid **(Pereira et al., 2017).**

- **Flavonoids:**

Flavonoids are universal within the plant kingdom; they are the most common pigments next to chlorophyll and carotenoids **(Stalikas, 2007).** They have a common biosynthetic origin and they all have the same basic skeleton **(Fig05)**, fifteen carbon atoms composed of two aromatic units, C6 cycles (A and B), linked by a C3 chain **(Zeghad, 2009).**

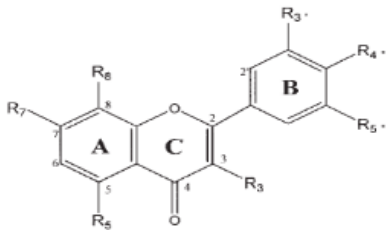


Figure 5: General structure of flavonoïde **(Crozier, 2003)**

Twenty-four flavonoids were identified in rosemary, and they are belonging to three main sub-classes: Flavones, Flavonols and flavonones **(Mena et al., 2016).**

- **Tannins:**

Tannins are complex phenolic compounds obtained from the condensation of simple phenols, they are metabolites classified in two groups according to their chemical structure and by their biogenetic, origin **(Fig06)**; hydrolysable tannins (carbohydrate ester and phenolic acids) and condensed tannins (dimers, oligomers and/or polymers of flavannes-3-ols or flavannes -3, 4-diols) **(Zemmouri, 2016).**

Studies have shown that rosemary extract contain gallic tannins. (**Fadili et al.,2015).**

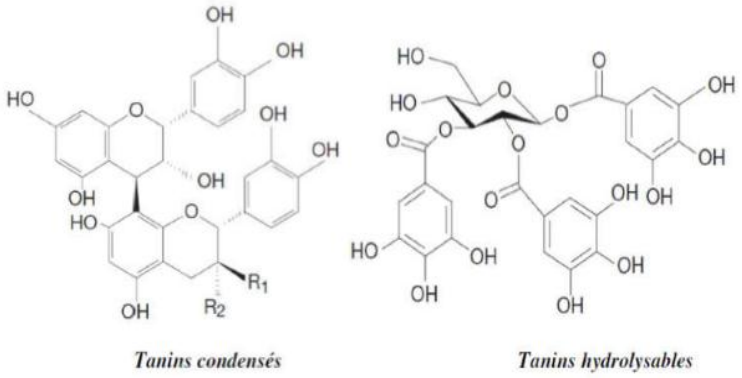


Figure 6: Typical chemical structures of tannins **(Achat, 2013)**

I.3 Pharmacological activities

Rosemary is constituted by bioactive molecules, the phytocompounds, responsible for implement several pharmacological activities; it has been used as an antispasmodic in renal colic and dysmenorrhea and in relieving respiratory disorders. It has also been used as an analgesic, antirheumatic, carminative, cholagogue, and diuretic, expectorant. *R. Officinalis* have also been identified such as an antifungal, antiviral, antibacterial, anti-inflammatory and antioxidant **(De Macedo et al., 2020).**

I.3.1 Antimicrobial activity of rosemary:

Several studies have proved the antibacterial and antifungal activities of rosemary. The anti-microbial activity of the plant is determined by the interactions between its components. Rosemary has been shown to inhibit the growth of bacteria, such as *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*, due to the action of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carsonic acid, rosmanol and isorosmanol. They interact with the cell membrane, generating changes in the genetic material and nutrients, and they also interact with the protein membrane and cause the loss of the membrane functionality and structure **(Nieto et al., 2018).** This impressive antibacterial activity makes *R. officinalis* a strong defense against common food pathogens and a promising new preservative that could replace artificial additives **(Tavassoli et al., 2011).** A study indicated that rosemary could even prevent the development of highly resistant fungal biofilms. A nanosystem was developed that would significantly block the adhesion and development of biofilms of Candida fungal strains **(Rasooli et al., 2008).** The results of a recent work **(Mousapour and Yassini 2020)** revealed that the number of microorganisms *(Escherichia coli, Staphylococcus aureus and Candida albicans)* in mayonnaise decreased in the presence of polypropylene films containing rosemary extract.

I.3.2 Antioxidant activity of rosemary:

Natural antioxidant products are increasingly being used to treat various pathological diseases associated with oxidative damage, including cancer, cardiovascular and neurodegenerative diseases **(Aherne et al., 2007)**, and they also widely used to retard undesirable changes as a result of oxidation in many foods **(Benincá et al., 2011)**. Reactive oxygen species, such as hydrogen peroxide and free radicals, like superoxide anion ($O^{\cdot-}$) and hydroxyl radical ($HO^{\cdot}$), are always produced as a result of metabolic processes or from external sources **(Botsoglou et al., 2010).**

Antioxidant activity of rosemary has received renewed attention and various studies *in vitro* have been done in the sense. The most pharmacological effect of rosemary is the consequence of high antioxidant activity of its main bioactive compounds; essential oil and phenolic compounds **(Rašković et al., 2014)**.While synergistic mechanisms between many components of the oil may contribute to the antioxidant activity, phenolic diterpenes such as carnosic acid, carnosol and rosmarinic acid have been determined to be the most potent antioxidants present in rosemary essential oil **(Zaouali et al., 2010).**

The rosemary biocompounds have been shown to be inhibitors of lipid peroxidation, it not only reduces the amount of reactive species in the organism, but also increases the activity of antioxidant enzymes **(Andrade et al., 2018)**; by limiting oxidative stress in the organism, rosemary helps to prevent and treat various pathological diseases associated with oxidative damage, including cancer, cardiovascular and neurodegenerative diseases **(Aherne et al., 2007).**

R. officinalis leaves are widely used as a condiment for flavoring food, and to retard undesirable changes as a result of oxidation in many foods **(Benincà et al., 2011**); addition of rosemary extract to sunflower oil of mayonnaise decreased the level of volatile compounds, formed from photooxidation in the headspace (**Lagunes-Galvez et al., 2002).** Another study of **Alizadeh et al. (2019)**, showed that rosemary essential oil had a powerful antioxidant effect in mayonnaise.

I.4 Extraction methods of antioxidant

The use of bioactive compounds in different commercial sectors such as pharmaceutical, food and chemical industries signifies the need of the most appropriate and standard method to extract these active components from plant materials. They can be extracted using a variety of methods. The most popular method of extraction is steam extraction, but as technological advances are made more efficient and economical methods are being developed. These include methods such as solvent extraction, supercritical fluid extraction, ultrasound and microwave extraction. (**Kabuba and Huberts, 2009).**

I.4.1 Microwave assisted extraction:

Microwaves (MW) are electromagnetic fields with frequency range from 300MHz to 300GHz. They are composed of two oscillating per-pendicular fields (magnetic field and electric field) **(Rehman et al., 2020)**. MAE relies on the contact of a dielectric polar substance and a fast-oscillating electric field produced by microwaves, which generates heat due to the friction caused by inter- and intramolecular movements **(Fig07)**. The heat induces the formation of water vapor in the cell, which eventually causes rupture and further leakage and release of intracellular components, led by an electroporation effect (**Chandra et al., 2020***)*. This technique has gained significant importance in the past few years owing to its "greener" and efficient extraction capacity.

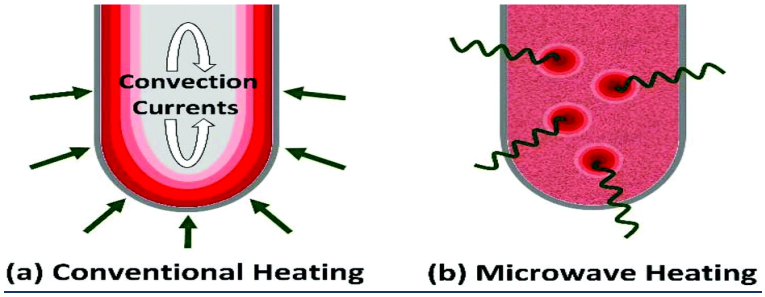


Figure 7: Conventional and microwave heating mechanisms **(Akbaian-Tefagh and Wile., 2018)**

I.4.2 Ultrasounds in Extraction processes:

Ultrasound frequency ranged between 20 kHz and 100 MHz travels through the medium by creating compression and expansion beyond the humanhearing. Therefore, ultrasound waves induce cavitation, thermal and mechanical effects in the extraction medium **(Fig08)** and disrupt the cell walls to enhance mass transfer without producing considerable changes in structure and properties of the targeted compounds **(Reddy et al.,2020),** as a result, the use of ultrasound in plant extraction is beneficial to increase mass transfer, better solvent permeability, less dependence on the solvent used, extraction at lower temperature, faster extraction rate and higher product yield **(Azmir et al., 2013).**

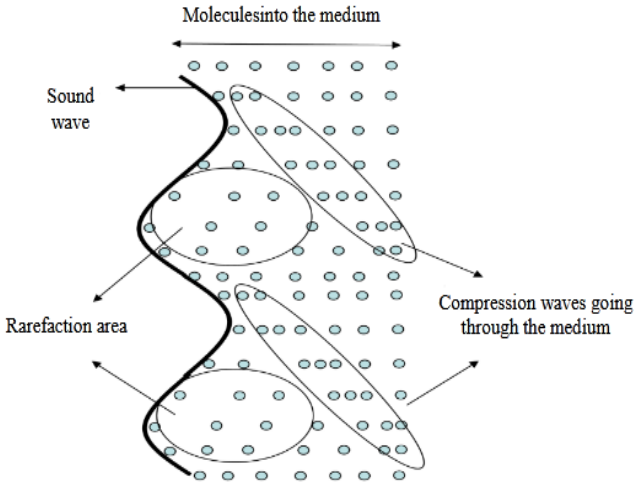


Figure 8: Compression and rarefaction cycles induced by a sound wave **(Achat et al., 2012).**

II. Mayonnaise

Mayonnaise is a semi-solid condiment. This emulsion includes an aqueous solution as a constant phase and oil as a dispersed phase. Widely consumed as a traditional seasoning due to its creamy mouth feel and special flavor. It's considered as microbe-stable food stuff due to its high fat content and acidic conditions **(Mirzanajafi-Zanjani et al., 2019).**

II.1 Composition of mayonnaise:

The ingredients used for the preparation of mayonnaise change according to the method of preparation. the traditional mayonnaise is prepared from simple ingredients **(Fig09)**, while at the industrial one is prepared from several ingredients, its produced by using vegetable oil, emulsifier (egg lecithin), acidic components (acetic acid, citric acid, and maleic acid), flavoring agents (sweetener, salt, mustard, or garlic), texture enhancers, stabilizers and an inhibitor for unwanted crystals **(Yildirim et al., 2016).**

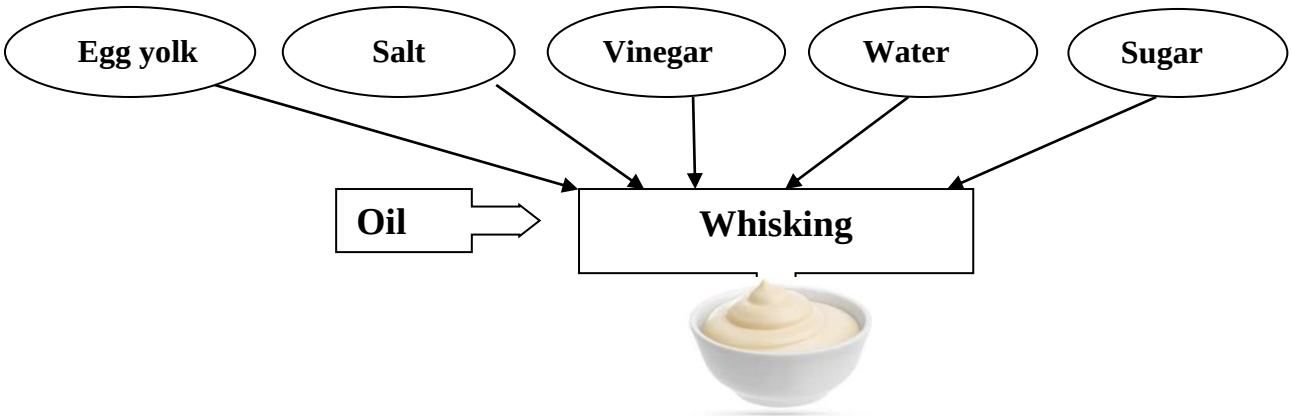


Figure 9: The ingredients used for the preparation of mayonnaise

II.2 Enrichment of mayonnaise:

In recent years, different studies have used edible oil and of mayonnaise as a solvent for extracting substances of interest from different plant matrices. **(Achat et al., 2012 ; Li et al., 2013 ; Penalvo et al., 2016 ; Flamminii et al., 2020 ; Achat et al., 2021).** Dried leaves and polyphenols of the plant are added to the water phase, during the preliminary mixing, which usually contains egg, vinegar, salt, sugar and water, **(Flamminii et al., 2020),** however the essential oil is added to the quantity of oil prepared for making

mayonnaise and they are thoroughly blended together with the water phase **(Teneva et al., 2020).**

Table below present examples of some works of enrichment of mayonnaise with antioxidants **(Tab III).**

Table III: Enrichment of mayonnaise with antioxidants

Extract	Conditions	Results	References
Grape seed extract (GSE)	0.5, 0.9 and 1.4 mg GSE per mL; stored in the dark at room temperature for 8 weeks.	The oxidative stability was improved in the mayonnaise enriched with GSE	**Altunkaya et al., 2013**
Olive leaf phenolic(OLE) Alginate/pectin + OLE	1g OLE /kg 4g Alg/pec + OLE /kg	-Improvement of the physical properties -low sensory acceptability	**Flamminii et al., 2020**
Fish oil	16%(v/v) in the final mayonnaise	better physical and oxidative stability of the mayonnaise	**Yesiltas et al., 2020**
Ferulago	1000ppm stored at ambient temperature for 6 months	Improvement of the oxidative stability of the prepared mayonnaise with ferulago extract	**Alizadeh et al., 2019**
Oregano essential oil (OEO)	0,2% (v/v) d'OEO	Reduction in the count of *Salmonella Enteritidis*	**Da Silva and Franco, 2012**
Essential oil of rosemary	450 ppm stored at ambient temperature for 6 months	Protective effects against primary and secondary changes in oxidation	**Alizadeh et al., 2019**
Rice bran *(Oryza sativa L.)*	Ethanolic extract (0.5%), Aqueous extract (2%), stored at 4 and 20^{o}C in the darkness and sampled after 7 days	Ethanolic extract has proven to be the most effective in both aspects, preventing the oxidation and the growth of some microorganisms	**Martillanes et al., 2019**

Material and methods

III. Material and methods

III.1 Chemicals

All solvents and reagents used were of analytical grade, 2, 2diphenyl-1-picrylhydrazil (DPPH°), was purchased from Sigma-Aldrich (Germany). Hexane, methanol, potassium chromate, silver nitrate, phenolphthalein, Hydroxyde de sodium (NaOH), ethanol, were supplied from Biochem-chemopharma (UK). Violet bile and Neutral Red (VRBL), Yeast Extract Glucose Chloramphenicol (YGC), Baird-Parker, Rappaport Vassiliadis Soy (RVS), Muller-Kauffmann broth, Xylose lysine désoxycholateb (XLD) and Hektoen agar, were purchased from Merck KGaA (Germany).

III.2 Material

Uv-vis spectrophotometer (Rayleigh, China), Microwave 23L (NN-S674MF. Maxi power, China), analytical balance, precision balance (Radwag, Poland), Water bath (Memmert, Germany), vortex mixer, pH meter, Ultrasonic cleaner (Bransonic, USA), Centrifuge (Hettich, Germany), Hand mixer (Robuste, China), Moisture analyser (Ohaus™), drying oven (Memmert, Germany), grinder (Ika a11 basic, Germany).

III.3 Plant material

The wild rosemary was collected at the flowering stage; it was collected in Aboudaou (Bejaia) on 28/02/2020. The geographical position of this region is: 36°38' 05.2n and 5° 13' 46.4s. After identification, the collected plant material was washed with running tap water to remove surface contaminants. The samples (leaves and flowers) were dried in a drying oven at 30°c to constant weight, then ground with a grinder; the powder obtained was sieved using a sieve with a pore diameter of less than 250 µm. The rosemary powder was stored in glass jars in the dark at room temperature.

III.4 Evaluation of moisture content

In order to determine the moisture content of the sample, the thermal drying method was used. 5g of the powder was placed in a 103^{o} ± 2C oven until a constant weight was obtained. The moisture content was calculated according to the following formula:

$$MC\% = Wi - Wo \setminus Wi \times 100$$

Where W0: correspond to the loss in weight (g) on drying and Wi: correspond to the initial weight of sample (g).

III.5 Extraction of rosemary bioactive substances

A preliminary study was done (in the previous work) in order to determine the type of extraction method for the rest of the investigation. In order to obtain the best yield extraction of rosemary bioactive substances (essential oil and phenolic compounds), several techniques were tested: micro-wave, ultrasounds, hydro-distillation, microwave with hydro-distillation, ultrasound-microwave assisted hydro-distillation (US-MAHD) and hydro-diffusion. Thus, microwave procedure was selected to the extraction of polyphenols and US-MAHD technique for essential oil extraction (already optimized in previous works).

III.5.1 Extraction of essential oil:

Microwave-assisted extraction was performed by employing a modified domestic microwave oven with cavity dimensions of 22.5 cm × 37.5 cm × 38.6 cm and 2450 kHz working frequency, the apparatus was modified in order to condense the steam generated during extraction into the sample **(Fig10)**. The condenser was connected to the low temperature bath circulator, refrigerated water, was circulated from low temperature bath circulator into the condenser to condense the vapor flowing from the microwave oven to the receiving flask **(Felkai-Haddache et al., 2015)**.

For the extraction of essential oil, 20g of rosemary leaves was stirred in 400ml distilled water, then submitted to sonication treatment. The mixture was irradiated using the microwave system; rosemary sample was heated using a fixed power of 700 W for 20min.

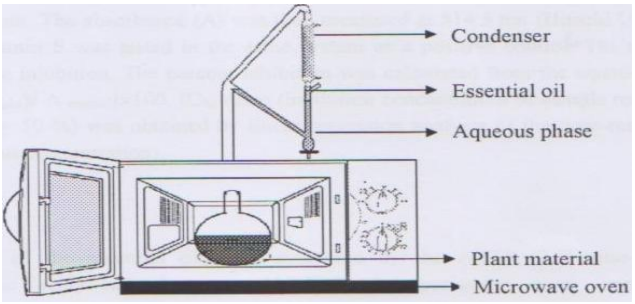


Figure10: Modified domestic microwave oven used for microwave-assisted extraction **(Mathialgan et al., 2014)**

The extraction yield of rosemary EO was obtained according to **Liu et al. (2011)**, and calculated following the equation: EOy = We/Wp x100

Where We: is the weight of the extract (essential oil), Wp: weight of the plant used for the extraction.

III.5.2 Polyphenols:

Using the same microwave system for EO extraction, the experiment was carried out in a round-bottom flask containing 1g of sample with 20ml ethanol (70%). The flask was set in a microwave stove and associated to condenser. The suspension was irradiated at 800 W, for 5 min. At the end of microwave irradiation, the volumetric flask was permitted to cool to room temperature. Then, the extract was filtered through Whatman No.1 paper, then centrifuged for 10 min at 5000 rpm (already optimized in previous works). The extract was collected in a volumetric jar until uses.

III.6 Formulation of mayonnaise at laboratory scale

Manufacturing of mayonnaise:

The preparation of mayonnaise was made in the laboratory of quality control (Cevital Agro-industry) respecting the diagram for making standard mayonnaise with addition of the vegetable matrix of rosemary. Thus, one kilogram of each mayonnaise sample was prepared in this study; the recipe contained the following ingredients in percentage (w/w): soybean oil (79%), egg yolks (6%), vinegar (5%), water (8%), salt (1%), and sugar (1%). All ingredients used were purchased from a local supermarket. Thus, egg yolks and sugar dissolved in water were mixed together then all other ingredients were added and stirred homogeneously by a hand mixer. The oil was poured in very slowly, while stirring **(Bouridane and Hamreulaine, 2018).**

In order to assess the addition effect of rosemary, as a functional ingredient, on mayonnaise quality, as a functional ingredient, five samples were prepared:

Sample (1): a control sample mayonnaise without any enrichment

Sample (2): mayonnaise + E.O (x ppm per liter of oil)

Sample (3): mayonnaise + rosemary leaves (x g/kg of product)

Sample (4) mayonnaise + polyphenols extract (x %)

Sample (5): Mayonnaise + rosemary powder (x g/kg of product)

Each sample was stored in two glass jars. One at room temperature (20° C) in the dark and the other in the refrigerator (4° C) for 30 days. Physicochemical, microbiological and antioxidant activity analyses were performed on all mayonnaise samples for 0, 15, and 30 days.

III.7 Microbiological analysis

Mayonnaises are relatively microbiologically unstable, and some ingredients, especially fresh egg yolk, are often contaminated. The viable populations of the principal groups of microorganisms were determined on mayonnaise samples, at production day, at 15 days after production, and at the end of storage (30 days).

The calculation of the number N of microorganisms per 10 g of product is obtained using the following equation:

$$N = \frac{\Sigma C}{n} \text{ CFU/10g}$$

ΣC: sum of the colonies counted on the retained boxes.

n: number of Petri dishes counted.

The Algerian regulation recommends the research of microorganisms listed in the **Table (IV),** governed by the Inter-ministerial Order of 2 Moharram 1438 corresponding to October 4, 2016 fixing the microbiological criteria of the food stuffs**.**

Table IV: Microbiological criteria of mayonnaise **(J.O N° 39 of 2 July 2017)**

Food categories	**Micro-organisms**	**Sampling plan**		**Microbiological limits (CFU/g)**	
		n	c	m	M
Unstabilized mayonnaise	Aerobic germs at 30°C	5	2	10^4	10^5
	Yeasts and molds	5	2	10^2	10^3
	Escherichia coli	5	2	10	10^2
	Coagulase-positive *Staphylococcus*	5	2	10^2	10^3
	Salmonella	5	0	Absence in 25g	
Stabilized mayonnaise	Yeasts and molds	5	2	10	10^2
	Escherichia coli	5	2	4	40
	Coagulase-positive *Staphylococcus*	5	2	10	10^2
	Salmonella	5	2	Absence in 25g	

- n: represents the number of units forming the sample, to be taken at random from a lot.

- m:represents the limit of the concentrations of microorganisms corresponding to a satisfactory hygiene of the processes considered, expressed as number of cfu per g or ml or cm².

-M: represents the limit of concentrations denoting unsatisfactory hygiene, usually expressed as number of cfu per g or ml or cm².

- c: represents the maximum allowed number of sample units.

III.7.1 Total coliforms

Culture media and reagents:

The medium used is crystal violet bile agar and neutral red (VRBL).

Procedure:

Prepare the stock solution by putting 10g of mayonnaise in 90g buffered peptone water with 0.7g of Tween 80.the mixture is then put in a water bath for 20 minutes.VRBL agar medium is also melted in a water bath. Briefly, 1ml of stock solution is poured into each petri dish, the VRBL agar is added and mixed carefully by slow rotation and left to solidify.The Incubation of total coliforms lasts 48h at 44°C. After incubation, the plates are counted.

III.7.2 Yeasts and molds

Culture media and reagents:

The medium used is Yeast Extract Glucose Chloramphenicol.

Procedure:

We have followed the same steps for the detection of total coliforms with incubation at 25°c for 5days.

III.7.3 *Staphylococcus aureus*

Culture media and reagents:

The medium used is Baird- Parker.

Procedure:

Prepare the stock solution by putting 10g of mayonnaise in 90g buffered peptone water with 0.7g of Tween 80.The mixture is then put in a water bath for 20 minutes. The medium is poured into Petri dishes and left to cool, then inoculated in streaks with 0.1ml of the stock solution and left to solidify. Incubation is performed at 37°C for 48 hours.

III.7.4 *Salmonella*

Procedure:

a- Pre-enrichment in non-selective liquid medium: Add 25 g of mayonnaise to 225 ml of buffered peptone water and incubateat 37°C for 24 hours.

b-Selective enrichment: Transfer 0.1 ml of pre-enrichment broth to 10 ml of Rappaport Vassiliadis Soy (RVS) Broth and incubate for 24 hours at 42° ± 1°C. At the same time inoculate 1ml of pre-enrichment broth to 10ml of Muller-Kauffmann broth. Shake and place in an oven at 37°C for 24h.

c-Selective isolation: Spread a 10 µl loop full from the inoculated and incubated Muller-Kauffmann broth and RVS broth on xylose lysine désoxycholate (XLD) and Hektoen agar plates during 24hours at 37°c.

III.8 Physico-chemical analysis:

The main physico-chemical factors analyzed on mayonnaise samples are: acidity test, salt content, pH and dry matter.

- pH:

The determination of the pH consists in the measurement of the acidity or the alkalinity of a product.

Procedure:

The pH measurement is carried out by a pH-meter by introducing the electrode beforehand calibrated into the sample to be analyzed; the value is displayed on the screen of the apparatus **(AFNOR., 1982)**.

- Salt content:

The determination of the salt content in a sample is done according to **MOHR (ISO 885/1.02.2004)** by titration with silver nitrate and potassium chromate as a color indicator, allowing first a reaction between silver ions and chlorine (Cl^-) which allows the formation of a silver chloride precipitate (Reaction 1).

$Na^{+}\ Cl^{--} + AgNO3 \rightarrow AgCl + NaNO3$ (1)

The end point of the titration occurs when all the chloride ions are precipitated. Then additional silver ions react with the chromate ions of the indicator, potassium chromate, to form a brick red precipitate of silver chromate (Reaction 2).

$K2CrO4 + 2\ AgNO3 \rightarrow 2\ KNO3 + Ag2CrO4.$ (2)

Procedure:

Weigh less than 1g of the sample into an Erlenmeyer flask; add 50-60ml of water at 55°C, then shake until the mayonnaise is dissolved in the water. Add 2 ml of 10% potassium chromate. Titrate with silver nitrate until the brick red color change is obtained. The salt content has been estimated as a percentage according to the following formula:

$$\textbf{Salt (\%)} = \frac{volum\ of\ titrated\ AgNO3 * 0.585}{m0}$$

m0: is the masse in the grams of the test sample.

-Acidity:

It is the acid-base titration with a sodium hydroxide solution NaOH, in the presence of phenolphthalein as a colored indicator.

Procedure:

In an Erlenmeyer, weigh 1 g of mayonnaise and add a quantity of water, shake so that the mayonnaise is dissolved in the water. Add two drops of phenolphthalein and titrate with NaOH (0.1N) until the pink color appears **(AFNOR., 1982)**. The acidity is estimated according to the following formula:

$$\textbf{Acidity (\%)}: \frac{volume\ of\ titrated\ NaOH * 0.6}{m0}$$

m0: is the masse in the grams of the sample.

- **Dry extract:**

Using an automatic dryer, equipped with a heating plate at 120°C and a balance.

Procedure:

According to the protocol used by Cevital agro industry, place an aluminum test pan in the desiccator and tare, spread out 5g of mayonnaise on the aluminum test pans, then place it in the machine until the result is displayed on the screen.

III.9 Antioxidant activity

Mayonnaise samples were submitted to the extraction method described by **Romeo et al. (2021)**: 2.5g of the mayonnaise was dissolved in 5 mL of methanol (60%) and 5ml of Hexane. the solution was vortexed then centrifuged at 3000 rpm for 10mn. The methanolic phase is collected and filtered, and the hexanic phase is centrifuged a second time with 5ml of methanol at 3000 rpm for 10mn. Thus, the second methanol phase is collected and filtered then added to the first one. The obtained extracts were evaluated for antioxidant activity by measurement of DPPH.

- **DPPH° assay:**

The antioxidant activity of sample extracts was measured by bleaching of the purple-colored solution of DPPH (2, 2-diphenyl-1-picrylhydrazyl) under reduction by an antioxidant compound.

The reaction can be summarized as the equation:

$$DPPH° + (AH) \longrightarrow DPPH\text{-}H + (A)n$$

Where: (AH) represents a compound capable of yielding hydrogen to the DPPH radical (purple) to transform it into diphenyl picryl hydrazine (yellow) **(Sánchez-Moreno, 2002).**

Procedure:

Approximately 0.1ml of the obtained extract was mixed with 1.9ml of the DPPH stock solution prior to incubation in the dark for 1hr. The absorbance was measured using

UV- spectrophotometer at 517 nm against a control **(Doulabi et al., 2020).** The inhibition percentage of DPPH°, radical scavenging activity (RSA), was calculated as:

$$\text{RSA (100 \%)} = \frac{Absconrol - AbsExtract}{Abscontrol} \times \mathbf{100}$$

Abs control: absorbance of DPPH° solution without any extract.

Abs Extract: absorbance of DPPH solution after reaction with the extract

III.10 Statistical analysis

All experiments were conducted in triplicate and results are expressed as mean ± standard deviation (SD). Statistical analysis was performed by analysis of variance, using the software GraphPad prism the differences are determined using tukey's test. Differences are considered to be significant at ($p<0.05$).

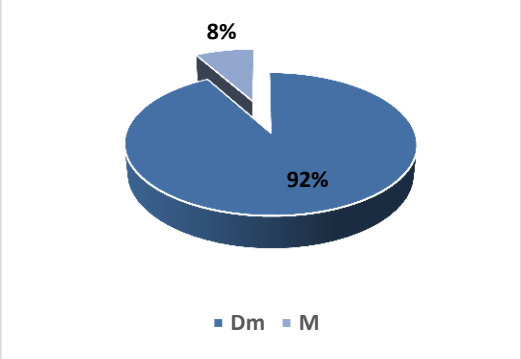
Results and discussion

IV. Results and discussion:

IV.1 Moisture content

The high concentration of moisture in fresh plant material leads to instability of some antioxidants or their degradation, sometimes caused by enzymatic action, as well as the growth of microorganisms that cause degradation of the plant matrix during storage. Dried plants retain high antioxidant capacity and total phenols because the enzymes have been inactivated due to decreased water activity**.** Drying is the most widely used treatment, inhibiting microbial growth and certain biochemical changes**. (Hossain et al., 2010)**

The results obtained from the moisture contents **(Fig11)** is about (8, 19±0.095 %) which is approximatively near than result (6.97%) reported by **Arslan and Musa Özcan. (2008).**



Dm: Dry matter; M: Moisture

Figure 11: Water and dry matter content of dried rosemary leaves

IV.2 Extraction of rosemary bioactive substances

IV.2.1 Extraction of essential oil:

The extraction of EO from plant tissues is usually performed using several classical methods such as steam distillation, hydrodistillation and liquid solvent extraction**,** although these techniques, they present many disadvantages, including large amounts of solvent, long extraction times or high energy consumption **(Okoh et al., 2010; Calinescu et al., 2014).** Recently microwave-assisted extraction has been the subject of lot of research. The popularity of the microwave technique is due to the rapid rates of heat transfer which

allows faster extraction times using less solvent and higher yields **(Zia et al., 2020).** Ultrasound has also been used to increase the efficiency of extraction of antioxidants from rosemary **(Albu, 2004).**

The yield of essential oil extracted by ultrasound microwave assisted hydro-distillation is about 0.4% which is lower than the result provided by **Aiche and Boubaya. (2019**) which is 2.4%. The difference between the extraction yield values of REO can be explained by the difference in the harvest period, soil, environement and part of plant.

IV.2.2 Extraction of polyphenols:

The mean value of extraction yield of rosemary plant using MAE is 0.38±0.01% of phenolic compounds (this result was obtained and estimated in previous work). Then the rosemary phenolic extract was directly used in our analysis

IV.3 Microbiological analysis

The **Table V** showed the evolution of the counts (CFU/g) at 0 days, 15 days and 30 days of microorganisms*: E. coli*, *Salmonella*, *S. aureus*, and yeasts and molds in mayonnaise samples.

Table V: Microbiological quality (Total number of bacteria, CFU/g) of mayonnaise samples during storage.

Storage (Days)	**Microorganisms (CFU/g)**	**Sample1**	**Sample2**	**Sample3**	**Sample4**	**Sample5**
1	*E.coli*	ND	ND	ND	ND	ND
	S. aureus	ND	ND	ND	ND	ND
	Salmonella	ABS	ABS	ABS	ABS	ABS
	yeasts and molds	44	ND	20	ND	ND
15	*E.coli*	ND	ND	ND	ND	ND
	S. aureus	ND	ND	ND	ND	ND
	Salmonella	ABS	ABS	ABS	ABS	ABS
	yeasts and molds	69	ND	10	ND	ND
30	*E.coli*	ND	ND	ND	ND	ND
	S. aureus	ND	ND	ND	ND	ND
	Salmonella	ABS	ABS	ABS	ABS	ABS
	yeasts and molds	80	ND	ND	ND	ND

ND: non-detectable; ABS: Absence in 25g

The results obtained revealed the absence of pathogenic bacteria; *Salmonella spp*. in 25 g of emulsion for all the samples from the beginning (Day 0) to the end of storage (30 days).

The data showed that *S. aureus* were not detected in all the analyzed samples during the storage. *E. coli* were not detected at day 0; 15 days or 30 days in anyone of tested samples however there was a growth of non-characteristic of *E. coli* colonies in the VRBL petri dishes of sample1 and their absence in the other samples.

Finally, molds and yeasts were quantified in samples 1 and 3 at the beginning of the experiment (44 CFU/g and 20 CFU/g respectively) and were not detected in the other samples (2,4,5). The number of these microorganisms was significantly reduced in sample 3 at day 15, and then no count was detected at day 30, while in the control there was an increase in the population (80 CFU/g) at the end of storage. For the other samples, yeasts and molds were not detected either at 15 or 30 days.

- All results were conform to microbiological criteria of mayonnaise, recommended by **J.O N° 39 of 2 July 2017**

Absence of *E. coli* in all samples can be due to the lack of contamination or to the low pH of the mayonnaise, however the absence of non-characteristic *E. coli* colonies in the other samples, indicates their inhibition by rosemary leaves and extracts. Indeed, rosemary has been shown to inhibit the growth of bacteria, such as *Escherichia coli* and *Staphylococcus aureus*, due to the action of its biomolecule contents; essential oil and polyphenols **(Nieto et al., 2018).**

The presence of yeasts and molds in sample 3 may be due to the concentration of the essential oil which is possibly not enough for a directly total inhibition. The results obtained in the present work confirm the data found by **Sacchetti et al. (2005).**

Garcia. (2006) claimed that mayonnaise was fairly resistant to microbial spoilage due to high-fat content and low pH. Mayonnaise is considered a microbiologically safe food product. Also, using vinegar to obtain a pH of 4.1 or less, consider the main reason for microorganism growth inhibition. In addition, the microbiological analysis results demonstrate that the use of rosemary or one of its extracts is effective in reducing undesirable germ contamination as well as yeast and mold in mayonnaise compared to the control.

IV.4 Physico-chemical analysis

-pH and acidity:

Physico-chemical measurements were carried out to study the behavior of mayonnaise in terms of pH and acidity during 30 days of storage **(Fig12, Fig13)**.

There is a significant difference in the pH values for each sample during the storage (D0, D15, D30), except sample 5 **(Appendix I)**. In all cases, it can be observed that the pH values decreased with time, the largest decrease in pH was observed for the sample without enrichment.

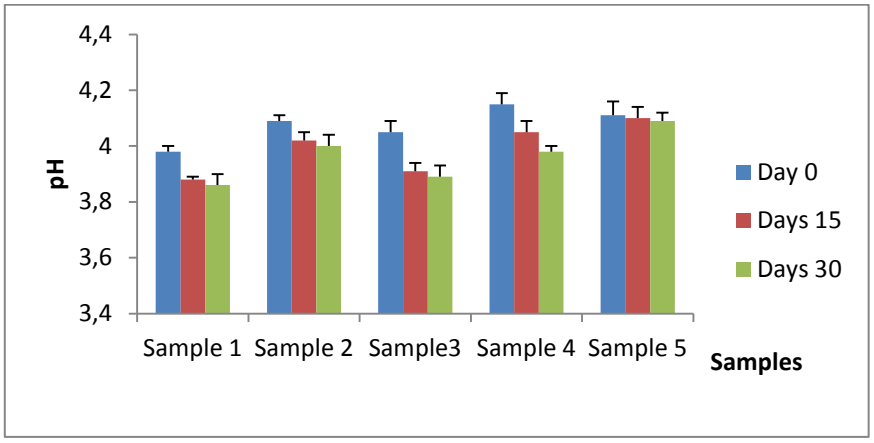


Figure 12: Evolution of pH during the storage in the dark at 20°C

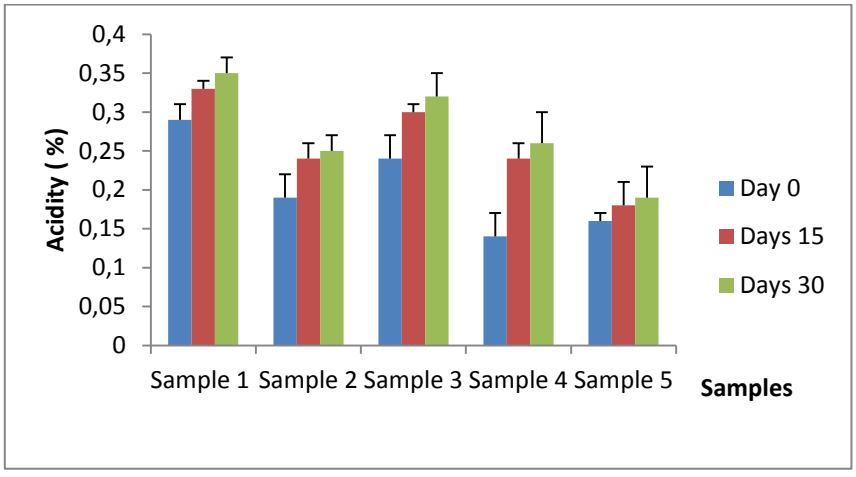


Figure 13: Evolution of acidity during the storage in the dark at 20°C

The acidity data of all mayonnaise samples revealed the same tendency of pH results.

The initial pH values of control mayonnaise compared with the results of sample 2, 4 and 5 were significantly different ($p< 0.05$) and no significant difference was found with sample3 **(Table VI).** However, at the end of storage (D30) significant differences were found among the pH results of sample 1 and the other samples, excluding sample 3 **(Table VIII)**. These variations of pH are probably due to a possible microbial growth which acidify the medium. Thus, the results show that rosemary permits to stabilize the pH to a less acidic degree.

TableVI: pH statistical analysis at Day 0

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Sample 1 vs. sample 2	0,1000	0,04711 to 0,1529	Yes	***	0,0003
Sample 1 vs. sample 3	0,05000	-0,002887 to 0,1029	No	Ns	0,0682
Sample 1 vs. sample 4	0,1500	0,09711 to 0,2029	Yes	****	<0,0001
Sample 1 vs. sample 5	0,1300	0,07711 to 0,1829	Yes	****	<0,0001

Table VII: pH statistical analysis at Day 15.

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Sample 1 vs. sample 2	0,09000	0,04781 to 0,1322	Yes	****	<0,0001
Sample 1 vs. sample 3	0,03000	-0,01219 to 0,07219	No	Ns	0,2336
Sample 1 vs. sample 4	0,09000	0,04781 to 0,1322	Yes	****	<0,0001
Sample 1 vs. sample 5	0,1500	0,1078 to 0,1922	Yes	****	<0,0001

Table VIII: pH statistical analysis at Day 30

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Sample 1 vs. sample 2	0,1000	0,03928 to 0,1607	Yes	**	0,0011
Sample 1 vs. sample 3	0,03000	-0,03072 to 0,09072	No	Ns	0,5627
Sample 1 vs. sample 4	0,09000	0,02928 to 0,1507	Yes	**	0,0029
Sample 1 vs. sample 5	0,1600	0,09928 to 0,2207	Yes	****	<0,0001

*, **, ***, ****, NS, ($p<0,05$), ($p<0,01$), ($p<0,001$) ($p<0,0001$) respectively, No significant

- Salt content (NaCl):

The salinity of the samples (**Fig.14)** presents a slightly decrease without a significant difference during the storage period **(Appendix III).**

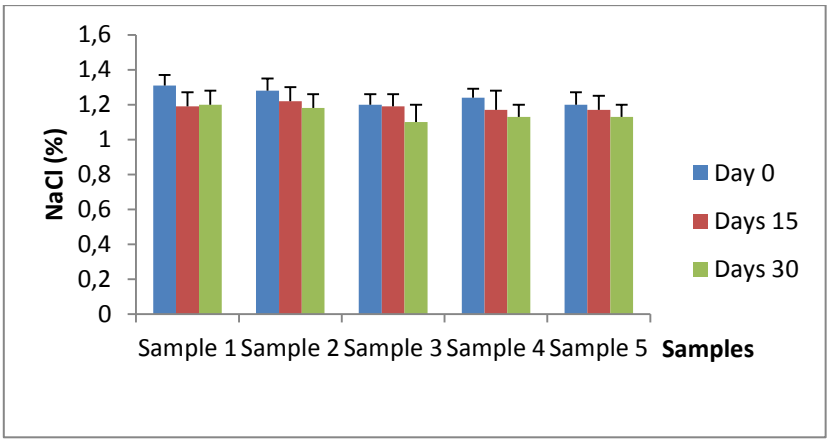


Figure 14: Evolution of NaCl during storage in the dark at 20°C

Table IX, X and XI showed that there is no significant difference between the salt content of all samples during the period of storage (30 days).

Table IX: NaCl statistical analysis at Day 0

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Sample 1 vs. sample 2	0,03000	-0,09065 to 0,1507	No	Ns	0,9360
Sample 1 vs. sample 3	0,1100	-0,01065 to 0,2307	No	Ns	0,0824
Sample 1 vs. sample 4	0,07000	-0,05065 to 0,1907	No	Ns	0,4133
Sample 1 vs. sample 5	0,1100	-0,01065 to 0,2307	No	Ns	0,0824

Table X: NaCl statistical analysis at Day 15

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Sample 1 vs. sample 3	-0,03000	-0,1949 to 0,1349	No	Ns	0,9787
Sample 1 vs. sample 3	0,000	-0,1649 to 0,1649	No	Ns	>0,9999
Sample 1 vs. sample 4	0,02000	-0,1449 to 0,1849	No	Ns	0,9954
Sample 1 vs. sample 5	0,02000	-0,1449 to 0,1849	No	Ns	0,9954

Table XI: NaCl statistical analysis at Day 30

Tukey's multiple comparisons Test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Sample 1 vs. sample 2	0,02000	-0,1346 to 0,1746	No	Ns	0,9940
Sample 1 vs. sample 3	0,1000	-0,05460 to 0,2546	No	Ns	0,3133
Sample 1 vs. sample 4	0,07000	-0,08460 to 0,2246	No	Ns	0,6380
Sample 1 vs. sample 5	0,07000	-0,08460 to 0,2246	No	Ns	0,6380

*, **, ***, ****, NS, (p<0,05), (p<0,01), (p<0,001) (p<0,0001) respectively, No significatif

The addition of salt to mayonnaise comes from the need to improve the palatability of the product; In addition, it slows down the development of certain micro-organisms and increases the shelf life.

- Dry extract:

From **Appendix IV** we can notice a significant difference in dry extract values for the mayonnaise samples 1, 2 and 3 during the storage (day 0, 15 and 30) and no significant difference for the two other samples 4 and 5.

Significant differences were recorded between the initial values of the control and all samples except sample 3 at the end of storage (**Table XII, XII and XIV).** It can be observed that dry extract values increased with time, but these differences were not significant ($p>0.05$) in sample 2 and 3.

The variation of these results (**Fig.15)** is probably due to the amount of different enrichment contained in the sampling.

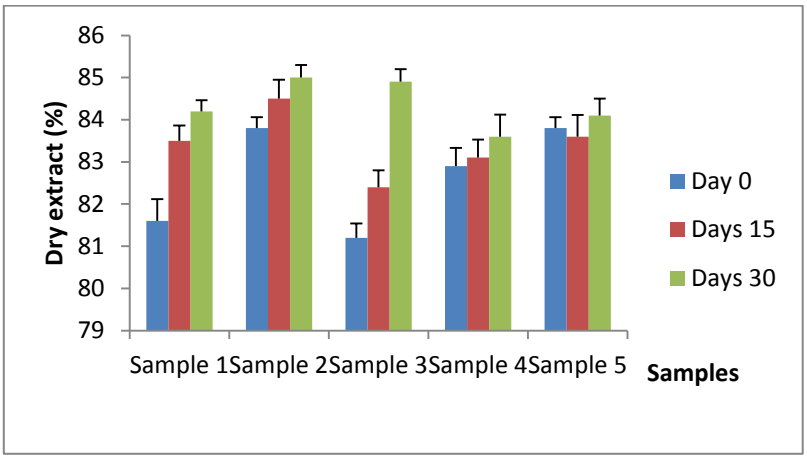


Figure 15: Evolution of dry extract during storage in the dark at 20°C

Table XII: Dry extract statistical analysis at Day 0

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Sample 1 vs. sample 2	-2,200	-2,881 to -1,519	Yes	****	<0,0001
Sample 1 vs. sample 3	0,4000	-0,2812 to 1,081	No	Ns	0,4021
Sample 1 vs. sample 4	-1,300	-1,981 to -0,6188	Yes	***	0,0002
Sample 1 vs. sample 5	-2,200	-2,881 to -1,519	Yes	****	<0,0001

Table XIII: Dry extract statistical analysis at Day 15

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Sample 1 vs. sample 2	-1,000	-1,781 to -0,2188	Yes	**	0,0095
Sample 1 vs. sample 3	1,100	0,3188 to 1,881	Yes	**	0,0044
Sample 1 vs. sample 4	0,4000	-0,3812 to 1,181	No	Ns	0,5303
Sample 1 vs. sample 5	-0,1000	-0,8812 to 0,6812	No	Ns	0,9943

Table XIV: Dry extract statistical analysis at Day 15

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Sample 1 vs. sample 2	-0,8000	-1,462 to -0,1377	Yes	*	0,0147
Sample 1 vs. sample 3	-0,7000	-1,362 to -0,03771	Yes	*	0,0359
Sample 1 vs. sample 4	0,6000	-0,06229 to 1,262	No	Ns	0,0851
Sample 1 vs. sample 5	0,1000	-0,5623 to 0,7623	No	Ns	0,9893

*, **, ***, ****, NS, ($p<0,05$), ($p<0,01$), ($p<0,001$) ($p<0,0001$) respectively, No significant

IV.5 Antioxidant activity:

In figures 16 and 17 are presented the results of the evaluation of the scavenging activity of the DPPH° radical, in mayonnaise enriched by different extracts of *R. officinalis* during 30 days of st orage at 4°C and 20°C. The test was repeated 3 times for each studied sample.

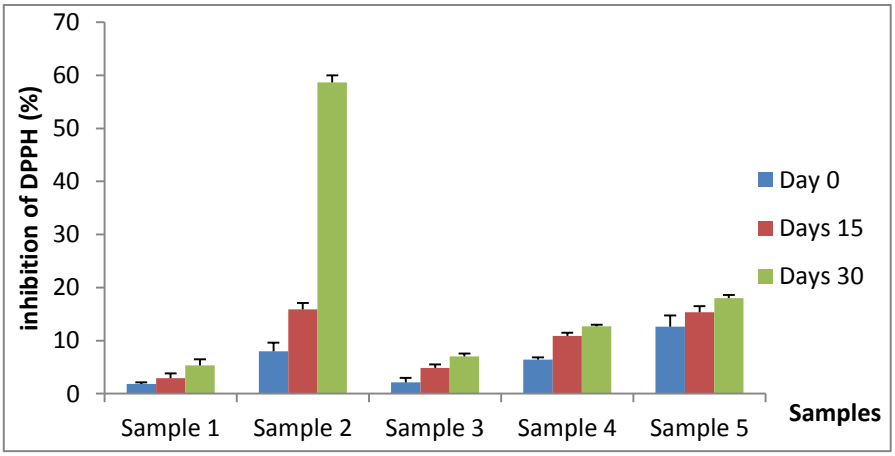


Figure 16: Antiradical-scavenging activity of the different samples of mayonnaise during storage in the dark at 20°C.

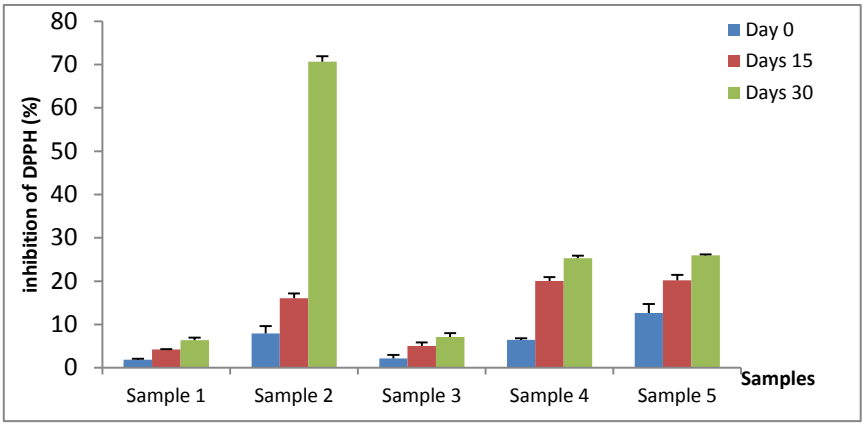


Figure 17: Antiradical-scavenging activity of the different samples of mayonnaise during storage at 4° C.

We notice in **Appendix V, VI** a significant increase in the trapping activity values ($p<0.0001$) of each sample during storage at 4°C and 20°C.

First of all, we observe at day 0 the values of scavenging activity of control sample and the other samples were significantly different ($p<0.0001$) expect sample 3 **(Table XV)**. After 15 days of storage, an increase in the scavenging activity is perceived in all samples at both of temperatures **(Table XVI, XVIII).**

Table XV: Statistical analysis of antiradical scavenging activity of mayonnaise at Day 0

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Sample 1 vs. sample	-6,103	-8,389 to -3,816	Yes	****	<0,0001
Sample 1 vs. sample 3	-0,2900	-2,577 to 1,997	No	Ns	0,9945
Sample 1 vs. sample 4	-4,588	-6,874 to -2,301	Yes	***	0,0001
Sample 1 vs. sample 5	-10,79	-13,08 to -8,506	Yes	****	<0,0001

Table XVI: Statistical analysis of antiradical scavenging activity of mayonnaise at Day15 at 20°C

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Sample 1 vs. sample 2	-13,00	-14,68 to -11,32	Yes	****	<0,0001
Sample 1 vs. sample 3	-1,940	-3,621 to -0,2588	Yes	*	0,0203
Sample 1 vs. sample 4	-8,000	-9,681 to -6,319	Yes	****	<0,0001
Sample 1 vs. sample 5	-12,45	-14,13 to -10,77	Yes	****	<0,0001

Table XVII: Statistical analysis of antiradical scavenging activity of mayonnaise at Day 30 at 20°C

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Sample 1 vs. sample 2	-53,32	-54,90 to -51,73	Yes	****	<0,0001
Sample 1 vs. sample 3	-1,695	-3,277 to -0,1128	Yes	*	0,0330
Sample 1 vs. sample 4	-7,355	-8,937 to -5,773	Yes	****	<0,0001
Sample 1 vs. sample 5	-12,67	-14,25 to -11,08	Yes	****	<0,0001

At 30 days of storage, the scavenging activity values for control sample and the other samples stored at 4° C increased significantly except sample 3 **(Table XIX).** The difference between the scavenging activity data for those stored at 20°C, are significant **(Table XVII).**

Table XVIII: Statistical analysis of antiradical scavenging activity of mayonnaise at Day15 at 4°C

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant ?	Summary	Adjusted P Value
Sample 1 vs. Sample 2	-11,86	-13,48 to -10,24	Yes	****	<0,0001
Sample 1 vs. Sample 3	-0,8800	-2,501 to 0,7409	No	ns	0,4758
Sample 1 vs. Sample 4	-15,89	-17,51 to -14,26	Yes	****	<0,0001
Sample 1 vs. Sample 5	-15,96	-17,58 to -14,34	Yes	****	<0,0001

Table XIX: Statistical analysis of antiradical scavenging activity of mayonnaise at Day 30 at 4°C

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Sample 1 vs. sample 2	-64,31	-65,70 to -62,92	Yes	****	<0,0001
Sample 1 vs. sample 3	-0,7600	-2,147 to 0,6267	No	ns	0,4670
Sample 1 vs. sample 4	-18,92	-20,30 to -17,53	Yes	****	<0,0001
Sample 1 vs. sample 5	-19,58	-20,97 to -18,19	Yes	****	<0,0001

*, **, ***, ****, NS, (p<0,05), (p<0,01), (p<0,001) (p<0,0001) respectively, No significant

There is a clear difference of an increase of antiradical acitivity between each sample. Among others, Sample 1 shows a very low antioxidant activity that can be related to the soybean oil used in the production of our mayonnaise.

According to the results, it can be seen that the DPPH radical scavenging activity of rosemary dried leaves in sample 2 is significantly higher than that of the different samples, this can be explained by a synergy between the essential oil and the polyphenols contents in the leaves.

The essential oil could not reach a 20% radical inhibition threshold even after one month of storage. This may be linked with the amount and the nature of the majority compounds of our essential oil which are determinant of the intensity of scavenging. These results can therefore confirm the data of different authors who report that the addition of rosemary extract to sunflower oil mayonnaise decreased the level of volatile compounds formed from photooxidation **(Lagunes-Galvez et al., 2002).** Rosemary extracts could have a chelating effect in sunflower oil mayonnaise.

Conclusion

The rosemary *R. officinalis.* L is a widespread and abundant species in Algeria. It is a cheap, available, and non-toxic herb that warrant the introduction of rosemary extracts and essential oils, with high phenolic compound contents, into the food industry. In this study, we were interested on the physico-

Chemical composition as well as the evaluation of the antioxidant and antimicrobial activity of polyphenols and essential oil extracted from dried leaves and powder of rosemary plant on mayonnaise.

Our study is divided into three parts;

The extraction of essential oil from leaves of *Rosmarinus officinalis.* L, using microwave extraction method (0.4%), was selected because it offers important advantages over traditional water-distillation and steam distillation, such as a shorter extraction time and better yields.

After preparing the mayonnaise samples with the different enrichments, a physico-chemical analysis was performed to determine the parameters influencing the product. The results obtained revealed that the pH values measured for all products are in the range {3.86±0.04-4.15±0.04}, all the samples analyzed have salt contents between {1.1%±0.1-1.31%±0.06} and dry extract contents between {81.2%±0.34- 85%±0.3}.

Tukey's statistical analysis revealed significant differences ($p<0.05$) in the pH test for mayonnaise enriched with dried leaves, powder and polyphenols, while for the dry extract, only mayonnaise with dried leaves and essential oil had significant differences. However, the rest of data presented no significant diferences; all mayonnaise enrichments in the Nacl test, mayonnaise with essential oil for the pH test and mayonnaise plus polyphenols and powder for dry extract.

The evolution results of the counts (CFU/g) at 0 days, 15 days and 30 days of microorganisms*: E. coli, Salmonella, S. aureus,* and yeasts and molds in mayonnaise samples, were conform to microbiological criteria of mayonnaise, recommended by **J.O N° 39 of 2 July 2017.** This analysis revealed also**,** that the use of rosemary or one of its extracts is effective in reducing undesirable germ contamination as well as yeast and mold in mayonnaise compared to the control.

The antioxidant capacity of the different 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 (70.69%±1.23), followed by rosemary powder (25.96%±0.26) and polyphenols extract (25.29%±0.58), finally the lowest percentage was attributed to essential oil (7.14%±0.85).

This modest work, revealed to be interesting at two levels. It allowed us to define a process of enrichment by rosemary in mayonnaise, but also to note an antioxidant, antimicrobial and antifungal activity of this plant. As perspective, it would be interesting to assess the quality of mayonnaise samples by evaluating, the stability and durability of the products, in the test "Rancimat"

The replacement of synthetic antioxidants by a natural plant extract can be healthier for human being and in the same time an important booster for our country's economy.

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Appendix

Appendix I: Statistical analysis of pH during stockage

Sample 1:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	0,1000	0,05441 to 0,1456	Yes	***	0,0005
D0 vs. D30	0,1200	0,07441 to 0,1656	Yes	***	0,0001

Sample 2:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	0,07000	0,01263 to 0,1274	Yes	*	0,0193
D0 vs. D30	0,09000	0,03263 to 0,1474	Yes	**	0,0045

Sample 3:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	0,1400	0,07552 to 0,2045	Yes	***	0,0005
D0 vs. D30	0,1600	0,09552 to 0,2245	Yes	***	0,0002

Sample 4:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	0,1000	0,03969 to 0,1603	Yes	**	0,0032
D0 vs. D30	0,1700	0,1097 to 0,2303	Yes	****	<0,0001

Sample 5:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	0,01000	-0,06503 to 0,08503	No	ns	0,9271
D0 vs. D30	0,02000	-0,05503 to 0,09503	No	ns	0,7445

Appendix II: Statistical analysis of acidity during stockage

Sample 1:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant ?	Summar y	Adjuste P Value
D0 vs. D15	-0,04000	-0,07604 to -0,00395	Yes	*	0,0310
D0 vs. D30	-0,06000	-0,09604 to -0,02396	Yes	**	0,0031

Sample 2:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-0,05000	-0,09745 to -0,00254	Yes	*	0,0396
D0 vs. D30	-0,06000	-0,1075 to -0,01255	Yes	*	0,0159

Sample 3:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	0,000	-0,05584 to 0,05584	No	ns	>0,9999
D0 vs. D30	-0,08000	-0,1358 to -0,02416	Yes	**	0,0079

Sample 4:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-0,1000	-0,1551 to -0,04494	Yes	**	0,0017
D0 vs. D30	-0,1200	-0,1751 to -0,06494	Yes	***	0,0005

Sample 5:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-0,02000	-0,07012 to 0,03012	No	ns	0,5297
D0 vs. D30	-0,03000	-0,08012 to 0,02012	No	ns	0,2675

Appendix III: Statistical analysis of Nacl during stockage

Sample 1:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	0,1200	-0,01195 to 0,2519	No	ns	0,0741
D0 vs. D30	0,1100	-0,02195 to 0,2419	No	ns	0,1026

Sample 2:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	0,06000	-0,07678 to 0,1968	No	ns	0,4691
D0 vs. D30	0,1000	-0,03678 to 0,2368	No	ns	0,1579

Sample 3:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	0,01000	-0,1210 to 0,1410	No	ns	0,9753
D0 vs. D30	0,08000	-0,05096 to 0,2110	No	ns	0,2553

Sample 4:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	0,07000	-0,07206 to 0,2121	No	ns	0,3925
D0 vs. D30	0,1100	-0,03206 to 0,2521	No	ns	0,1319

Sample 5:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	0,02000	-0,1441 to 0,1841	No	ns	0,9386
D0 vs. D30	0,06000	-0,1041 to 0,2241	No	ns	0,5831

Appendix IV: Statistical analysis of dry extract during stockage

Sample 1:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-1,900	-2,545 to -1,255	Yes	****	<0,0001
D0 vs. D30	-2,600	-3,245 to -1,955	Yes	****	<0,0001

Sample 2:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-0,7000	-1,266 to -0,1339	Yes	*	0,0179
D0 vs. D30	-1,200	-1,766 to -0,6339	Yes	***	0,0006

Sample 3:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-1,200	-1,766 to -0,6339	Yes	***	0,0006
D0 vs. D30	-3,700	-4,266 to -3,134	Yes	****	<0,0001

Sample 4:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-0,2000	-0,9561 to 0,5561	No	ns	0,7477
D0 vs. D30	-0,7000	-1,456 to 0,05608	No	ns	0,0690

Sample 5:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	0,2000	-0,4581 to 0,8581	No	ns	0,6840
D0 vs. D30	-0,3000	-0,9581 to 0,3581	No	ns	0,4439

Appendix V: Statistical analysis of scavenging activity during stockage at 20°C

Sample 1:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-1,038	-2,447 to 0,3718	No	ns	0,1546
D0 vs. D30	-3,453	-4,862 to -2,043	Yes	***	0,0002

Sample 2:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-7,935	-10,21 to -5,657	Yes	****	<0,0001
D0 vs. D30	-50,67	-52,94 to -48,39	Yes	****	<0,0001

Sample 3:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-2,688	-3,820 to -1,555	Yes	***	0,0003
D0 vs. D30	-4,858	-5,990 to -3,725	Yes	****	<0,0001

Sample 4:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-4,450	-5,138 to -3,762	Yes	****	<0,0001
D0 vs. D30	-6,220	-6,908 to -5,532	Yes	****	<0,0001

Sample 5:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-2,695	-5,031 to -0,3594	Yes	*	0,0256
D0 vs. D30	-5,325	-7,661 to -2,989	Yes	***	0,0003

Appendix VI: Statistical analysis of scavenging activity during stockage at 4°C

Sample 1:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-2,338	-2,953 to -1,722	Yes	****	<0,0001
D0 vs. D30	-4,518	-5,133 to -3,902	Yes	****	<0,0001

Sample 2:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-8,095	-10,28 to -5,907	Yes	****	<0,0001
D0 vs. D30	-62,73	-64,91 to -60,54	Yes	****	<0,0001

Sample 3:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-2,928	-4,257 to -1,598	Yes	***	0,0004
D0 vs. D30	-4,988	-6,317 to -3,658	Yes	****	<0,0001

Sample 4:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-13,64	-14,65 to -12,62	Yes	****	<0,0001
D0 vs. D30	-18,85	-19,86 to -17,83	Yes	****	<0,0001

Sample 5:

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
D0 vs. D15	-7,505	-9,821 to -5,189	Yes	****	<0,0001
D0 vs. D30	-13,31	-15,62 to -10,99	Yes	****	<0,0001

*, **, ***, ****, NS, (p<0,05), (p<0,01), (p<0,001) (p<0,0001) respectively, No significatif

Abstract:

The objective of this study was to evaluate the antimicrobial and antioxidant activity of the different samples of mayonnaise enriched with rosemary plant (dried leaves, powder, essential oil and polyphenols), as well as their influence on the physicochemical parameters of the mayonnaise during storage (30 days).

The results obtained showed that the enriched mayonnaise recorded less physicochemical changes than the control (mayonnaise without enrichment).

The different enrichments allowed to obtain a better microbiological quality of the mayonnaise,which is reflected in absence of the researched bacterial strains, *E. coli, S. aureus, Salmonella* as well as yeasts and molds.

The mayonnaise sample enriched with dried leaves revealed an antioxidant activity with the best antiradical capacity (70.69%±1.23).

Tukey's statistical analysis revealed significant differences ($p<0.05$) in the pH test for mayonnaise enriched with dried leaves, powder and polyphenols, while for the dry extract, only mayonnaise with dried leaves and essential oil had significant differences.

The data showed that rosemary extracts can be used as a natural antioxidant and antimicrobial agent with a better stability of mayonnaise quality.

Keywords: Mayonnaise, Rosemary, Physico-chemical, Antimicrobial, Antioxidants.

Résumé :

L'objectif de cette étude était d'évaluer l'activité antimicrobienne et antioxydante des différents échantillons de mayonnaise enrichis en romarin (feuilles séchées, poudre, huile essentielle et polyphénols), ainsi que leur influence sur les paramètres physicochimiques de la mayonnaise pendant le stockage (30 jours).

Les résultats obtenus ont montré que la mayonnaise enrichie a enregistré moins de changements physicochimiques que le contrôle (mayonnaise sans enrichissement).

Les différents enrichissements ont permis d'obtenir une meilleure qualité microbiologique de la mayonnaise,qui s'est traduit par l'absence des souches bactériennes recherchées, *E. coli, S. aureus, Salmonella* ainsi que des levures et moisissures.

L'échantillon de mayonnaise enrichi en feuilles séchées a révélé une activité antioxydante avec la meilleure capacité antiradicalaire (70,69%±1,23).

L'analyse statistique de Tukey a révélé des différences significatives ($p<0,05$) dans le test du pH pour la mayonnaise enrichie en feuilles séchées, en poudre et en polyphénols, tandis que pour l'extrait sec, seule la mayonnaise avec les feuilles séchées et l'huile essentielle présentait des différences significatives.

Les données ont montré que les extraits de romarin peuvent être utilisés comme un antioxydant naturel et un agent antimicrobien avec une meilleure stabilité de la qualité de la mayonnaise.

Mots clés : Mayonnaise, Romarin, Physico-chimique, Antimicrobien, Antioxydants.