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MASTER

Thème

Formulation d'un lait fermenté aromatisé au Lentisque.

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DEDICATION

For those which gave me everything without anything in return

There are no words to describe how much my parent has meant to me throughout all my life.

Mom and Dad, you have given me so much, thanks for your faith in me, and for teaching me that I should never give up. Thanks for lending me your ear on countless occasions when I needed to vent my frustrations…

To my sisters and brother Ryma, Massinissa and Mayssa Without you, my life would fall apart; you have always been there for me with encouraging words.

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To my grandparents, *uncles and aunts, and cousins.*

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To all my faithful friends, you have a big place in my life.

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I dedicate this work:

In memory of my father

Mom, you have given me so much, thanks for your faith in me, and for teaching me that I should never give up. Thanks for lending me your ear on countless occasions when I needed to vent my frustrations …

To My brother Achour chichiou

To my Grandparents

To my uncles and aunts spécialy Cherif and Feriel

To my frends Hakima, Sylia, Saida, Sofiane

To all my friends and colleagues

Lynda

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List of Acronyms

ANOVA: Analysis Of Variance

BHA: Butylated hydroxyanisole

EO: Essential Oil

EC_{50} : Effective Concentration

IC: Inhibitory Concentration

Mo : phosphomolybdenum

NonC: Non contable

OD : Optic Density

RC_{50}: Reducing concentration.

RSA: Radical Scavenging Activity

TAC: Total antioxidant capacity

TPC**:** Total Phenolic Compounds

VRBL: Violet Red Bile Agar

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Introduction

Introduction:

In spite of modern improvements in slaughter hygiene and food production techniques, food safety is an increasingly important public health issue. It has been estimated that as many as 30% of people in industrialised countries suffer from a food borne disease each year and in 2000 at least two million people died from diarrhoeal disease worldwide. There is therefore still a need for new methods of reducing or eliminating food borne pathogens, possibly in combination with existing methods**. (Burt 2004)**

Aromatic plants have been widely used to extend the shelf life of foods and in folk medicine. It is known that most of their properties are due to the essential oils (Eos) that they contain as products of their secondary metabolism. **(Adam, Sivropoulou et al. 1998).**

One of the modern ways to improve the hygienic safety of manufactured food products is to exploit the antimicrobial properties of natural plant extracts, allowing for the reduction of the use of chemical antimicrobial agents, which constitute a potential human health hazard. In this regard, the antimicrobial properties of EOs have been known for a long time and continue to be the subject of several studies that evaluate their microbial potential as alternatives to chemical agents in Food industries (**Djenane, Yangüela et al. 2013)**

The essential oil of mastic tree (*Pistacia lentiscus L.*) has been shown to have antibacterial, anti-fungal, insecticides and antioxidants effects. Mastic tree is also used in cosmetics, perfumes and as a flavoring in food preparations (**Haloui, Farah et al. 2015**). Flavored mastic fermented milk is known ancestrally in Kabylia (North of Algeria). The role of fermented milk in human nutrition is well documented and the virtues of these products were known to man even during the ancient days of civilization. These products have long been an important component of nutritional diet. (**Panesar 2011**). In recent years, specific attention has been given to the adoption of selected traditional fermented dairy products in some countries for technology transfer to small and medium industrial scale. Such development in food processing has alleviated hunger in some countries of the world and enhanced the level of contribution of traditional fermented methods of manufacture to food safety and sustainable economic development **(Benkerroum and Tamime 2004).**

Manufacturing of a mastic flavored fermented milk by adding the essential oil has been traditionally inspired, therefore the aim of this study is the evaluation of flavoring a dairy product “Leben” for eventual preservation. For this, biological activities (antioxidant and antimicrobial activities) of lentisk essential oil were estimated, then a flavoured “Leben” was formulated at laboratory scale, finally assessment of the incorporation of this essential oil in “Leben”: Impact on physicochemicals and microbiolgicals parameters.

Bibliography

I- Overview of *Pistacia lentiscus* plant:

Plants represent an extraordinary reservoir of new preventives and curatives molecules. The most important elements are alkaloids, flavonoids, vitamins, tannins, essential oils, organic acids, resins, fat oils, saponins and polysaccharides **(Rojas, Hernandez et al. 1992, Rawani, Pal et al. 2011)**. The Mediterranean region is relatively rich with plants(15,000 - 20,000 species) **(Bhattacharjee, Chatterjee et al. 2011)** and *Pistacia lentiscus* L, an evergreen dioecious shrub, is widely distributed along the basin shores (**Zohary 1952)**.

P. lentiscus has an economic value as it is the source of a traditional medicinal agent. It is used as a food ingredient in the Mediterranean region. It has been effective in the treatment of benign gastric ulcers. There have been various studies of *P. lentiscus*, especially after the discovery of the biological activities of the essential oils **(Djenane, Yangueela et al. 2012).**

In Algeria, the tree is widespread in forest alone or associated with other tree species such as terebinth, olives and carob, in all coastal areas up to 700 m above sea level or in seaside stony areas (**Fig.01)**. It succeeds in any ordinary garden soil, preferring a hot dry position in full sun. It prefers a well-drained to dry sandy or stony alkaline soil, making it more abundant near the sea. It also shows tolerance to rocky areas, drought and cold (−7 ◦C) in winter together with resistance to calcareous soil and re-growth after cutting fire injuries **(Yıldırım 2012).**

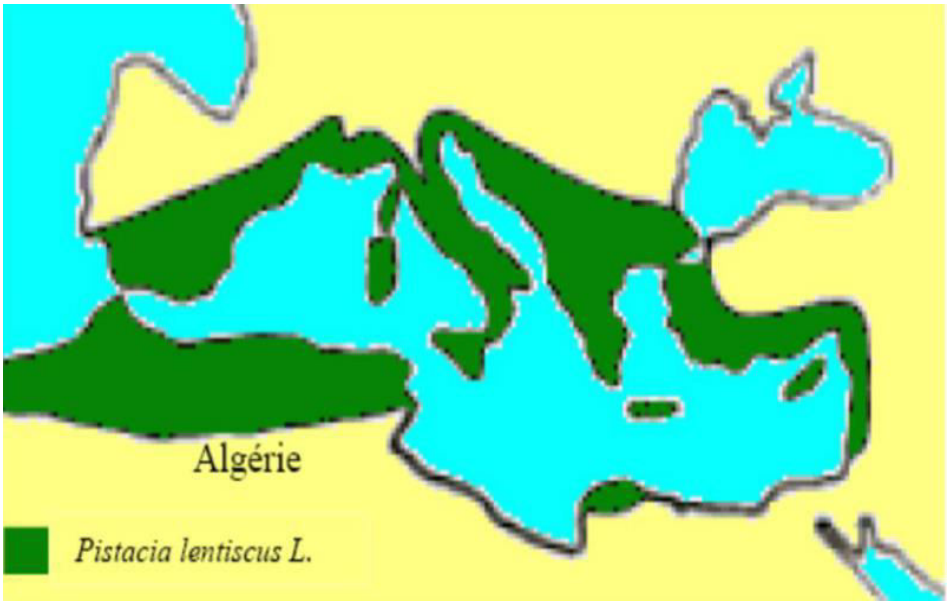

Figure 01: Distribution area of *Pistacia lentiscus* in the Mediterranean basin (**Seigue 1985)**

- The processing of the mastic tree can lead to the production of three main products: mastic gum, pressed oil extracted from mastiha berries and the essential oil (EO) from flowers, leaves and branches **(Barra, Coroneo et al. 2007).**

Vernacular names

English: Mastic tree (Lentisk), French: Arbre à mastique (Lentisque), Berber: Amaḍaɣ Arabic: الضرو, Spanish: Lentisco.

I-1- Morphological description:

Pistacia lentiscus L. (lentisk) is an evergreen bush that can reach 3 m in high, belonging to the Anacardiaceae family that consists of more than eleven species (**Fig.02**) **(Presti, Sciarrone et al. 2008)**. The genus *Pistacia* is characterized by its dioecious reproductive system (male and female plants) and by its homeochlamydic perianth of flowers **(Mabberley 1997).** The *Lentiscus* section includes the evergreen species with paripinnate (no terminal leaflet) leaves and smaller seeds **(Parfitt and Badenes 1997**).

Flowering takes place between Mid March and the end of April. Male inflorescences aggregate 8–10 flowers. The female flower has one seminal primorde. Flowers on male trees are deep red, and on female trees, yellow. Fruits are ripe occurs 150–230 days after the onset of flowering **(Jordano 1989**). The fruit is a drupe, first red and then black when ripe, about 4 mm in diameter. *P. lentiscus* seeds are dispersed by birds.

Kingdom: Plantae
Division: Magnoliophyta
Order: Sapindales
Family: Anacardiaceae
Genus: Pistacia
Species: *Pistacia lentiscus*.
Binomial name: *Pistacia lentiscus L.*

Figure 02: Taxonomic classification of *pistacia lentiscus L.*(**Nahida and Siddiqui 2012**)

I-2- Chemical compositions of lentisk:

P. lentiscus L. is a rich source of essential oils (96 components) **(Presti, Sciarrone et al. 2008**), condensend and hydrolysable tannins **(Abbas M., Boudriche D. 2007)**, glycosides flavonoïques (**Vaya and Mahmood 2006**), anthocyanins **(Longo, Scardino et al. 2007),** resin « mastic of chio » **(Leonti et al, 2001**), triterpenes (**Atmani et al, 2002**), fatty acids such as oleic, palmitic and linoleic acids, representing the 50.72%, 23.16% and 21.75% of the lipid fraction, respectively and polyphenols (**Trabelsi, Cherif et al. 2012**).

I-3-Essential oils:

Essential oils (EO) are complex mixtures of volatile organic compounds produced as secondary metabolites in plants; they are constituted by hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols and phenol ethers). They frequently are responsible for the distinctive odor of plants. **(Nerio, Olivero-Verbel et al. 2010)**.

- **Terpene hydrocarbons:**

Terpenes are the most common class of chemical compounds found in essential oils **(Fig.03).** They are made from isoprene units (several 5 carbon base units, C5), which are the combinations of 2 isoprene units, called a "terpene unit." Essential oils consist of mainly monoterpenes (C10) and sesquiterpenes (C15), which are hydrocarbons with the general formula (C5H8)n. The diterpenes (C20), triterpenes (C30), and tetraterpenes (C40) exist in essential oils at low concentration **(Mohamed, El-Emary et al. 2010).**

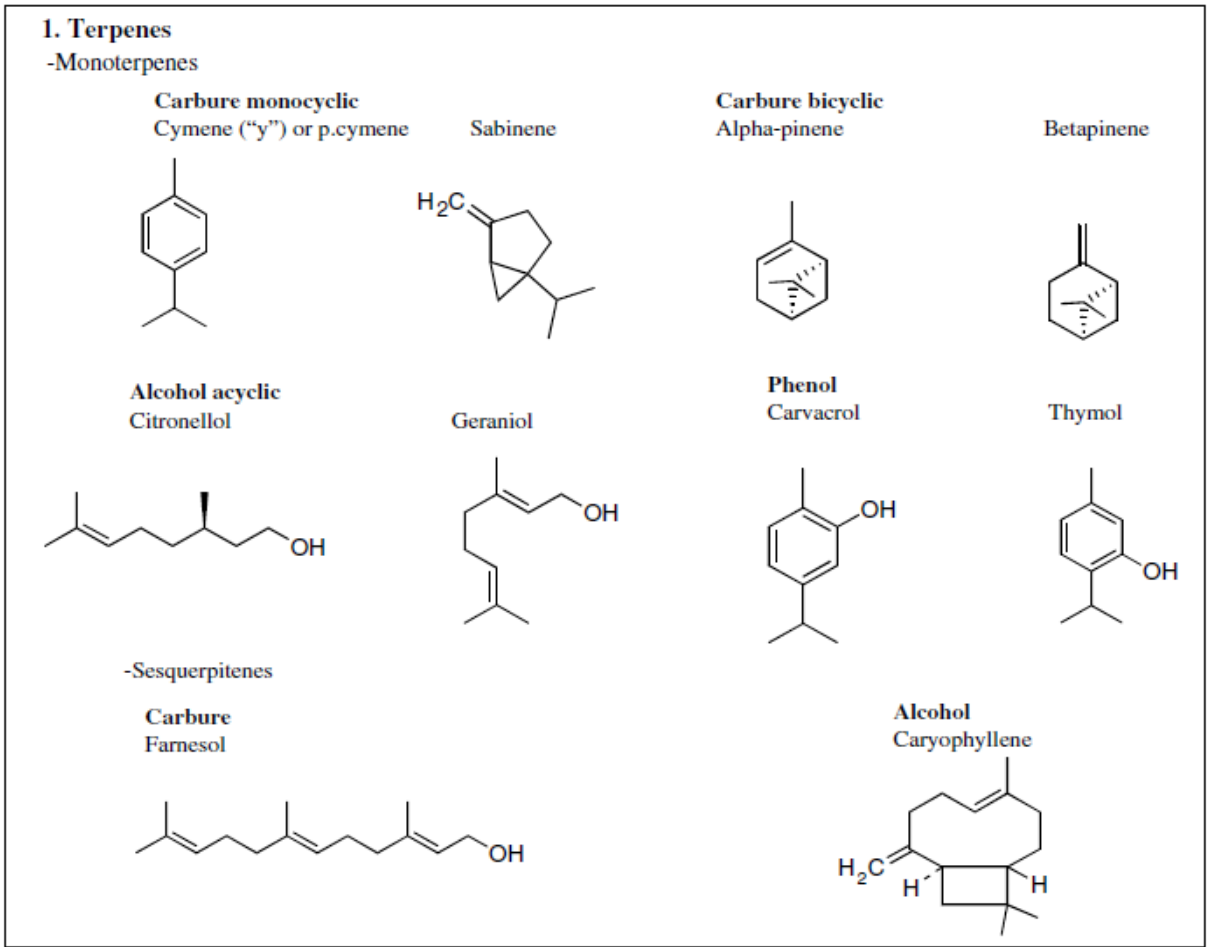

Figure 03: Chemical structures of terpenes essential oils **(Bakkali, Averbeck et al. 2008)**

- **Oxygenated compounds**

Derived from phenylpropane, the aromatic compounds occur less frequently than the terpenes. These molecules are the combination of C, H, and O, and there are a variety of compounds found in essential oils **(Fig.04)**. Oxygenated compounds can be derived from the terpenes, in which they are termed "terpenoids" **(Burt 2004**).

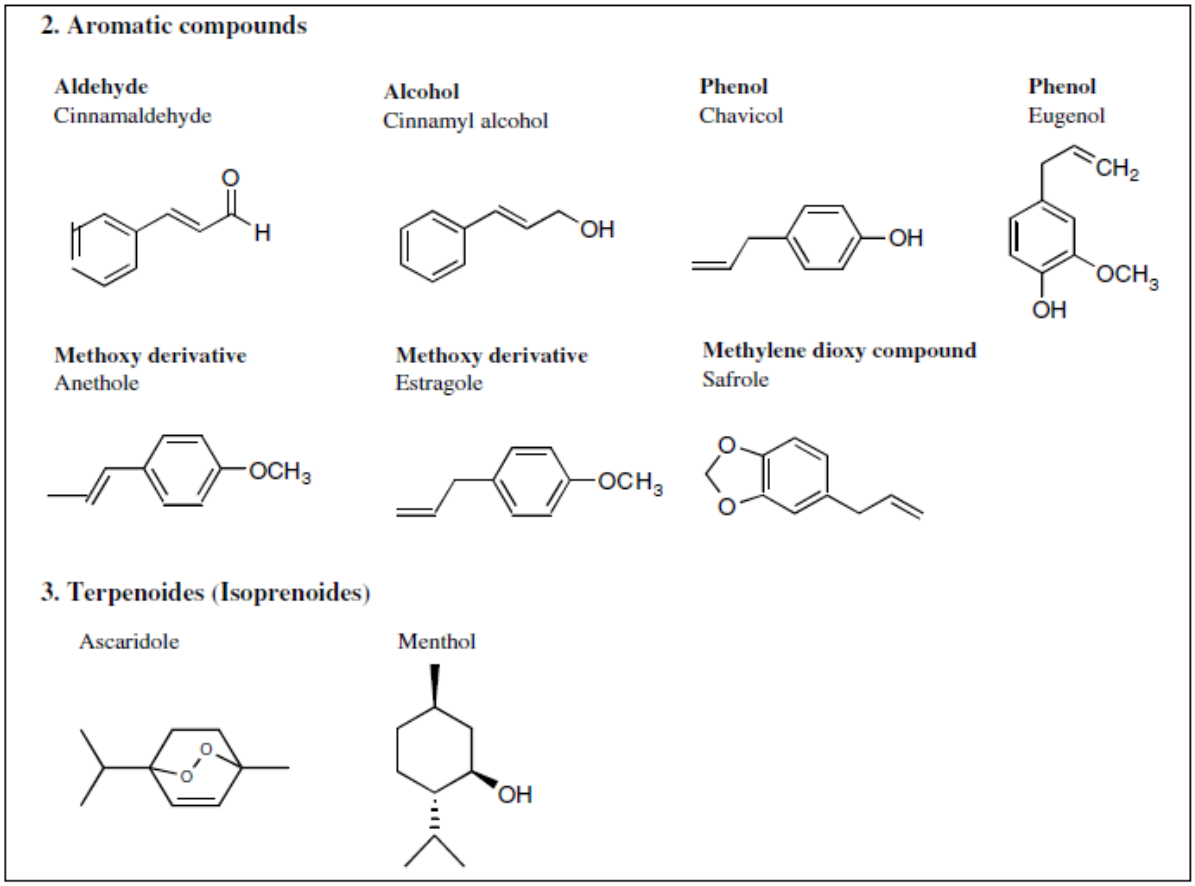

Figure 04: Chemical structures of aromatic components of essential oils (**Bakkali, Averbeck et al. 2008**)

- Some researchers have reported the chemical composition of the essential oil from leaves of *P. lentiscus* of diverse origins**, (Table 1 and 2)**. The chemical composition of the *P. lentiscus* EO was also analyzed using a gas chromatography mass spectrometry (CG-MS) technique.

Table 01: Essential oils of Algerian *Pistacia lenticus* **(K. Arab 2014)**.

Compounds	**Chemical composition**	**% Rate**
α-Pinène	$C_{10}H_{16}$	1,665
R-Comphéne	$C_{10}H_{16}$	0,290
β-Pinène	$C_{10}H_{16}$	1,100
β-Mircene	$C_{10}H_{16}$	1,255
α-Phellandrene	$C_{10}H_{16}$	0,605
α-Terpinène	C_6H_{10}	0,424
Limonène	$C_{10}H_{16}$	4,760
γ-terpinène	C_6H_{10}	0.828
L-terpinen-4-ol	$C_{10}H_{18}O$	3,832
3-cyclohexene-1-methanol,α-α 4-trimethyl		3,736
Carvone		
L-α-bornyl acetate	$C_{10}H_{14}O$	2,805
1-(3-methylcyclopent-2-enyl) cyclohexene	$C_{12}H_{20}O2$	1,192
Undecanone	$C_{12}H_{18}$	0.733
Caryophyllen	$C_{11}H_{22}O$	5,580
β-Cubebene	$C_{15}H_{24}$	3,219
Spathulenol	$C_{15}H_{24}$	5,539
α-Cadinol	$C_{15}H_{24}O$	13,353
	$C_{15}H_{26}O$	4,112

Table 02: Chemical analysis of essential oils of lentisk around the Mediterranean basin

Origin	Major components	References
Spain	α-Pinene and myrcene	**(Boelens and Jimenez 1991)**
Greece	α-pinene 58.9–70%	**(Papageorgiou, Mellidis et al. 1991)**
Corcia	tepinene-4-ol and α-pinene	**(Castola, Bighelli et al. 2000)**
Israel	α-terpineol	**(Fleisher and Fleisher 1992)**
Egypt	d-3-carene 65%	**(De Pooter, Schamp et al. 1991)**
Sardinia	Terpinen-4-ol 22 %	**(Picci, Scotti et al. 1987)**
France	α-pinene 16%	**(Buil, Garnero et al. 1975)**
Tunisia	α-pinene 17%	**(Douissa, Hayder et al. 2005)**

I-3-1- Extraction of essential oils:

Essential oils can be extracted using a variety of methods, although some are not commonly used today. Currently, 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, and microwave extraction. The suitability of extraction method varies from plant to plant and

there are significant differences in the capital and operational costs associated **(Kabuba and Huberts 2009).**

I-3-1-1-The conventional method:

The Clevenger consists of a main tube combined with condenser and graduated tube with glass stopcock. A return tube for the aqueous part of the distillate connects the bottom of the measuring tube and the main tube. Eighty grams of fresh and clean **(Basma, Asrar et al. 2013**). *P. lentiscus* leaves were placed into the three necked round extraction in flask and soaked with water. Clevenger apparatus was linked to the flask . The flask was heated using heating mantel. Water and leaves, were mixed and allowed to boil. Water and extracted lentisk oil evaporate. The vapour mixture condensed using reflux condenser, from condenser distillate water and oil flow in to gradated tube; as the oil is not miscible with water it may be easily separated and started accumulating and distillate water returning to the flask, after the oil has been separated from it, so that can be re-boiled. The oil was allowed to stand for sufficient time, to be clear and then it was collected and stored in dark glass vial in a refrigerator until it has been tested (**Basma, Asrar et al. 2013)**.

I-4- Biological activities of essential oils of lentisk:

The essential oil of leaves of *Pistacia* species has been the object of several studies of their antibacterial and antioxidant activities **(Tassou and Nychas 1995, Douissa, Hayder et al. 2005, Benhammou, Bekkara et al. 2008)**

I-4-1- Antimicrobial activity:

The crude alocoholic extract obtained from the leaves of *Pistacia lentiscus L.* has reported to inhibited the growth of *Phythium ultimum* and *Rhizoctania solani fungus* significantly and further study revealed that all extract are more effective on *P. ultimum* than *R. solani* **(Ali-Shtayeh and Abu Ghdeib 1999)**. Essential oil from aerial parts which contain terpineol and α-terpineol was also found to be effective against mycelian growth of *A. flavus* **(Benhammou, Bekkara et al. 2008)**

- *Pistacia lentiscus* L. has found to be effective against *Staphylococcus aureus* and *E. coli* and it also has antimycotic activity **(Ali-Shtayeh and Abu Ghdeib 1999, Magiatis, Melliou et al. 1999).** It's essential oil which is obtained from, twigs and mastic gum by steam distillation showed *in vitro* antimicrobial activity (**Hayder, Ben Ammar et al. 2005)** and antifungal activity against *Rhizoctania solani* **(Congiu, Falconieri et al. 2002)**. It's aqueous and flavonoid enriched extract and essential oil from leaves has marked inhibitory effect against

Salmonella typhi murium and lower inhibitory effect on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella enteritidis* (**Aksoy, Duran et al. 2006)**

I-4-2- Antioxidant activity:

Essential oil which was collected at flowering stage contain high monoterpene hydrocarbon fraction (45-68.35%), showed highest free radical scavenging activity and antioxidant capacity **(Assimopoulou, Zlatanos et al. 2005, Barra, Coroneo et al. 2007, Kottakis, Kouzi-Koliakou et al. 2009)**. Natural resin and bioactive triterpenes from essential oil also, showed antioxidant property so these are used in functional food due to this property **(Bhouri, Derbel et al. 2010)**

I-4-3- Other pharmacological actions:

As hypotensive effect due to procyanidine (**Sawidis, Yurukova et al. 2010)**, as aphrodiasac **(Dabos, Sfika et al. 2010)**, in the treatment of functional **dyspepsia (Qiao, Li et al. 2011)** as antiasthmatic in allergic asthma by inhibiting eosinophilia and reducing airway hyper-responsiveness and suppressing the production of inflammatory cytokines (IL-5 and IL-13) as well as chemokines (eotaxin and eotaxin2) in broncho alveolar lavage fluid **(Kaliora, Stathopoulou et al. 2007**) in chron's disease **(Kaliora, Stathopoulou et al. 2007),** anthelmitic activity **(Manolaraki, Sotiraki et al. 2010).**

I-5- Traditional uses of lentisk:

Since ancient times, *Pistacia lentiscus L.,* are known as part of the traditional pharmacopoeia of several Mediterranean countries **(Abdeldjelil, Bensegueni et al. 2014**). Aqueous extract of *P. lentiscus* L. leaves is a very popular drink in North Africa countries and is becoming increasingly popular worldwide, partly because of more documented evidence about its beneficial health properties **(Trabelsi, Cherif et al. 2012).** The fruits, galls, resin and leaves of the *P. lentiscus* have a long tradition in folk medicine dating from the times of the ancient Greeks. In Algeria the oil of the fruit is used by the population in traditional medicine in many ways, as an antidiarrhoeal and also as constituent of cattle feed. **(Charef, Yousfi et al. 2008)**

- Mastic is still used in traditional folk human and veterinary medicine around the Mediterranean basin. Relative to humans, mastic gum and leaf infusions were deemed good for prevention of digestive problems, useful for bronchitis, teeth sanitation and against jaundice, bedwetting, and headaches caused by colds. Veterinary uses encompass treatment of ectoparasites, wound healing (external application of leaf and fruit oil), bloating and

bellyaches (leaves, ingested per os) and even disinfection of water wells by using foliage brooms **(Landau, Muklada et al. 2014).**

- Lentisk oil may partially help in the protection against mercury intoxication, and it could also be considered a safe nutritional source, at least by maintaining total cholesterol and LDL-cholesterol in their normal ranges **(Maarouf, Cherif et al. 2008)**.

- The essential oil of Lentisk is extensively used in the perfumery and in food and pharmaceutical industries as reported by **(Calabro and Curro 1974).** It has been re-evaluated as a flavouring in alcoholic beverages and chewing gum **(Fernandez, Camacho et al. 2000)**..

I-6- Food additives:

I-6-1- Definition:

According to Codex Alimentarius (**Alimentrius** 2015); food additive means any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result (directly or indirectly), in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include contaminants or substances added to food for maintaining or improving nutritional qualities.

I-6-2- Use of additives:

The use of food additives is justified only when such use has an advantage, does not present an appreciable health risk to consumers, does not mislead the consumer, and serves one or more of the technological functions set out by (**Alimentrius 2015)**, and the needs set out from (a) through (d) below, and only where these objectives cannot be achieved by other means that are economically and technologically practicable:

a) To preserve the nutritional quality of the food; an intentional reduction in the nutritional quality of a food would be justified in the circumstances dealt with in sub-paragraph (b) and also in other circumstances where the food does not constitute a significant item in a normal diet;

b) To provide necessary ingredients or constituents for foods manufactured for groups of consumers having special dietary needs;

c) To enhance the keeping quality or stability of a food or to improve its organoleptic properties, provided that this does not change the nature, substance or quality of the food so as to deceive the consumer;

d) To provide aids in the manufacture, processing, preparation, treatment, packing, transport or storage of food, provided that the additive is not used to disguise the effects of the use of faulty raw materials or of undesirable (including unhygienic) practices or techniques during the course of any of these activities.

I-6-3- Use of essential oils as natural food preservatives:

A number of EO components have been registered by the European Commission for use as flavorings in foodstuffs. The flavorings registered are considered to present no risk to the health of the consumer. United States Food and Drug Administration (FDA) has classified the substances as generally recognized as safe (GRAS) or as approved food additives. An overview of the literature reporting studies on the antibacterial effect of EOs or their components in foods (fresh meat, meat products, fish, milk, dairy products, vegetables, fruit and cooked rice), have shown that the concentration needed to achieve a significant antibacterial effect. **(Burt 2004)**

I-7- Fermented milk "Leben":

I-7-1- "Leben" between tradition and industry:

Fermented milks with different names and ripened by micro-organismes under similar conditions are available in most countries (**Mahmoud and Kosikowski 1982**). The role of starter cultures in the manufacture of fermented dairy products is to provide microbiologically safe products with recognizable organoleptic and structural properties in an efficient and reproducible way **(Weerkamp, Klijn et al. 1996**). The traditional "Leben" is generally processed from cow's milk. It is the most popular traditional product known in the North Africa and the Middle East. (**Samet-Bali, Ayadi et al. 2014).** "Leben" is a refreshing cultured product obtained by spontaneous fermentation of cow's milk. Occasionally, goat's milk alone or in combination with cow's milk is used; however, the same product is made in different Arab countries and it is known as lben or leben (in North African countries) and laban (in the Middle East). **(Benkerroum and Tamime 2004)**

- Flavored fermented milk mastic is known ancestrally in Kabylia (North of Algeria). The Kabyle woman churn her milk in a squash at least that is how it happened before. Before incorporating the milk inside, squash is rubbed with the leaves of the mastic tree and then the

cap is secured with some branches of the mastic tree. This tradition still exists, but especially in rural and mountain environment. Kabyle woman sits and shakes strongly squash that is hooked to the ceiling beam of the house or outside a branch. The woman grabbed the gourd from the sides through ropes or netting that surrounds the squash.

- "Leben" is also produced on an industrial scale as fermented pasteurized milk. The acidification is caused by inoculation of mesophilic lactic ferments. It undergoes pasteurisation at 84 ° C for 30 seconds, then cooled to 22 ° C, seeded with lactic bacteria (*Streptococcus cremoris* , *Streptococcus lactis* and *Streptococcus diacetylactis*, *Leuconostoc dextranicum , Ln. citrovorum and Ln. mesenteroides*) (**Benkerroum and Tamime 2004**). The physico-chemical composition of "Leben" is variable; average values for the principal components are shown on table 03 **(Tantaoui-Elaraki and El Marrakchi 1987).**

Table 03: Physico-chemical composition of "Leben"

Parameters	Value
pH	4.2
Titrabe acidity	8.2
Fat	8.9 g/l
Lactic acid	8.2 g/l
Total protein	25.6 g/l
Lactose	26.9 g/l
Total dry mater	89 g/l

- According to the available literature, it can be suggested that the health benefits associated with fermented milks consumption is well known for centuries. Fermented milk is considered as healthy food due to its high digestibility and bioavailability of nutrients and also can be recommended to alleviation of lactose intolerance, protection against gastrointestinal infection, anticarcinogenic effect, immune system stimulation, lowering of serum cholesterol, alleviation of constipation, antihypertensive activity, and antiallergenic qualities **(Panesar 2011).**

Material and methods

II- Materials and methods

II-1- Chemicals

All solvents and reagents used were of analytical grade. Sodium carbonate ($N_{a2}CO_3$),, 2, 2-diphenyl-1-picryl-hydrazil (DPPH) , trichloroacetic acid extra pure ($C_2HCl_3O_2$) were purchased from Sigma-Aldrich (Germany). Sodium acetate anhydrous ($C_2H_3N_aO_2$ $3H_2O$) and potassium chloride (KCl) ,Iron (III) chloride hexahydrate ($Fecl_{3,}6H_2O$) were supplied from Biochem-chemopharma (UK), ammonium molybdate tetrahydrate ($H_{24}Mo_7N_6O_{24}$, $4H_2O$) was purchased from Biochem-chemopharma(Canada) ,potassium ferricyanide [$K_3F_e(CN)_6$] was supplied from Biochem-chemopharma(USA).

II.2. Plant material:

Pistacia lentiscus leaves were collected in "Taza Jijel national park". Plant material was dried at room temperature away from light for 3 days to determine the moisture and the yield was expressed relatively to the dry matter. Essential oils obtained were stored in opaque glass bottles at 4 °C **(Haloui, Farah et al. 2015**).

Microbial strains:

The microbial strains studied are the same that contaminate the fermented milk "Leben ", they were removed from petri dishes seeded with the same type of contaminated fermented milk.

II-3- Biological activities of lentisk essential oil

II-3-1- Antioxidant activity:

II-3-1-1- Radical-scavenging test:

The radical-scavenging activity of *Pistacia lentiscus* essential oil was evaluated by the DPPH° assay. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable highly colored free radical that can abstract labile hydrogen atoms from phenolic antioxidants with concomitant formation of a colorless hydrazine (DPPH-H). The free radical-scavenging activity (RSA) of an extract can be expressed as the percentage of DPPH reduced by a given amount of extract. The free RSA was measured, following ***(Achat, Tomao et al. 2012)***. Briefly, an aliquot of the studied essential oil was subjected to four serial dilutions. 1 ml of each solution was added to 2 ml of DPPH solution (4×10^4mol/L in methanol) and the mixture was left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm Uv-vis light spectrophotometer (UV-VIS Spectrophotometer UV-9200, Biothech Engineering Management CO.,Ltd. UK). The quantity (µg) of dry extract per mL of reaction medium necessary to decrease the initial DPPH radical concentration by 50% (EC_{50}) was determined

using an exponential curve. The total RSA of each sample was expressed as the percentage of DPPH reduced and was calculated by the following equation:

$$\text{RSA} = \left(\frac{A_0 - A}{A_0}\right) \times 100$$

A_0: Absorbance of DPPH solution without any antioxidant; A: Absorbance of DPPH solution after reaction with the extract. All experiments were performed in triplicate.

II-3-1-2- Reducing power:

The ability of lentisk essential oil to reduce iron (III) was determined according to (**Kadri, Chobba et al. 2013**). Each sample (1–5 mg ml^{-1}) in ethanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide, and the mixture was incubated at 50 °C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 1500 g for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionised water and 1 ml of 0.1% ferric chloride, and the absorbance was measured at 700 nm against a blank Uv-vis light spectrophotometer (UV-VIS Spectrophotometer UV-9200, Biothech Engineering Management CO.,Ltd. UK). A higher absorbance indicates a higher reducing power. EC_{50} (mg ml^{-1}) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis. Antioxidant α -tocopherol was used as control and all tests were carried out in triplicate.

II-3-1-3- Total antioxidant activity:

The total antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of **Gokturk Baydar et al.. 2007.** This method is based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex by sodium sulfide. The obtained phosphomolybdenum blue complex is oxidized by the addition of nitrite and this causes a reduction in intensity of the blue colour.Briefl y, a 0.1 mL of sample aliquot was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tubes were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expressed as the absorbance of the sample. The phosphomolybdenum assay of BHA was also assessed for comparison.

II-3-2- Antimicrobial screening:

II-3-2-1- Antibacterial activity:

The antibacterial activity of *Pistacia lentiscus* essential oil was measured by a diffusion test (CA-SFM, 2009) using Mueller–Hinton agar previously inoculated with 1 mL of 18 h old of 10^6 CFU/mL bacterial suspensions of Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*. Sterilized paper discs (6 mm) were impregnated with 20 µL of different concentrations of essential oil dissoleved in dimethylsulfoxide (DMSO), serially diluted, and placed onto nutrient agar. The plates were incubated at 4 ◦C for 2 h to allow diffusion of the active compounds in the medium **(Tagg and McGiven 1971)**. DMSO was served as the negative control. Incubation of plates was performed at 37 ◦C for 24 h. The antibacterial activity was expressed as the diameter of inhibition zones produced by the samples against test microorganisms. The experiment was repeated in triplicate and the mean of diameter of the inhibition zones was calculated

II-3-2-2- Antifungal activity:

The antifungal agar disc diffusion method was used to assess the antifungal activity of *Pistacia lentiscus* essential oil ((Touaibia 2015). Young cultures of each strain were used to determine the optical density (OD) of the fungal suspension at 630 nm wavelength, in order to standardize the spore suspension10^7spores/ml. The OD of 0.04 corresponds to a concentration of 10^7 spores / ml.

Inoculating plates, 9 cm in diameter, containing Sabouraud agar medium supplemented with chloramphenicol from suspensions containing 10^7 spores/ml. 100 µl of this inoculums were then added. Incubation of plates was carried out at 30 °C for 15 to 20 minutes.
Sterilized paper discs (6 mm) were impregnated with 20 µL, 30 µL, 40 µL and 60 µL,,of essential oil, and incubation at 30 °C for 3 days. Each assay was performed three independent times in triplicate.

II-4- Formulation of flavoured fermented milk “Leben” at laboratory scale

II-4-1- Manufacture of flavoured Leben:

The preparation flavoured fermented milk “Leben” was made in the laboratory of the dairy industry “SARL RAMDY” (Bejaia, Algeria), respecting the diagram for making

standard "Leben" , with addition of *Pistacia lentiscus* essential oil, under aseptic conditions.(Fig.)

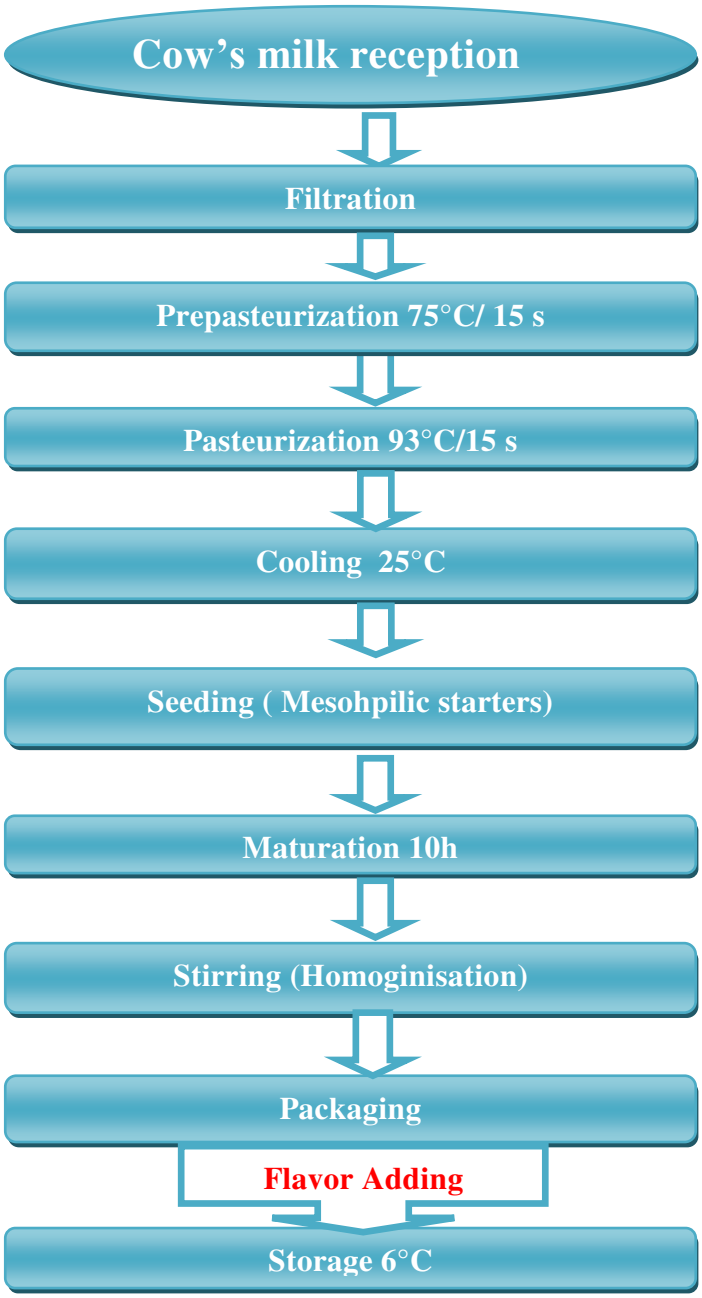

Figure 05: Flow chart for the manufacture of flavored leben.

II-4-2- Physico-chemical properties of "Leben":

Physico-chemical properties of the manufactured "Leben" were determined namely, pH, the dry extract and fat contents.

Table 04: Physico-chemical properties of **"Leben"**

Measure	Method
pH	The pH value of "Leben" was measured at fixed temperature (20 °C) with a calibrated pH electrode (HANNA HI 2210).
Total dry extract %	2 g of "Leben" placed in After 19 minutes; the result of drying is displayed on screen of the device as a weight percentage of dry matter relative to the total.
Fat contents %	The Gerber method: Milk fat is separated from proteins by adding sulfuric acid and using amyl-alcohol then centrifugation. The fat content is read directly via a special calibrated butyrometer.

II-4-3- Microbiological analysis:

Microbiological quality of prepared "Leben" was evaluated by enumerating total viable organisms. The organisms include total mesophilic aerobic bacteria, yeast, moulds, fecal coliforms, *Staphylococcus aureus* and salmonella. These were done according to procedures outlined in JORA **(Tab 05).**

Table 05: Microbiological analysis of manufactured "Leben"

Micro-organisms	Selective mediums	Incubation temperature	Incubation time	Method
Total flora	Plate Count Agar	37°C	48h	Leben" was prepared aseptically, using decimal dilutions, 1ml of each "Leben" samples in 9 ml sterile distilled water. 1ml of dilutions was spread plated in triplicates into prepared and dried petri-plates of suitable media for the enumeration of different organisms
Staphylococcus aureus	Baird parker	37 °C	48h	
Fecal coliforms	VRBL	30 °C	24h	
Salmonella	MacConkey	37 °C	48h	
Yeasts & moulds	Saboraud	25°C	5 days	

VRBL: Violet Red Bile Agar

II-4-4- Incorporation of lentisk essential oil in "Leben": Impact on physicochemicals and microbiolgicals parameters:

II-4-4-1- Effect of essential oil on pH and acidity of "Leben":

Different concentration of *Pistacia lentiscus* essential oil (0.04%, 0.02 %, 0.01% and 0.005%), were added to the prepared "Leben", in order to assess their influence on acidity and pH parameters. The measurements were carried out from day 0 (D 0) until expiration date and more.

Table 06 : Acidity and pH of the manufactured "Leben"

Measure	Method
pH	The pH value was measured with a calibrated pH meter (HANNA HI 2210).
Dornic acidity (°D)	10 ml of "Leben" was titrated with sodium hydroxide (N/9) with some drops of phenolphthalein, as a color indicator, until apparition of the pink color.

II-4-4-2- Antibacterial effect of essential oil on contaminated Leben:

The study of the antibacterial effect of lentisk essential oil on contaminated "Leben", was performed by adding different concentrations of this essential oil to the contaminated fermented milk, with two types of microorganism: *Staphylococcus aureus* and *Escherichia coli*.

- **Method:**

The aim was to inoculate bacterial strains, on the dairy product "leben". The bacterial inocula concentrations were evaluated by turbidity and were expressed by measuring the OD at 600 nm, until obtained an OD 0.08-0.1 which corresponds to 10^8CFU / mL (**Haddouchi, Lazouni et al. 2009**). Then 1 ml of *Pistacia lentiscus* essential oil was incorporated in 9 ml of contaminated leben, serial of decimal dilutions were prepared, in order to obtain four concentrations of essential oil (10 %, 1% , 0.1 % , 0.01 %) .

The enumeration of viable *S. aureus* and *E.coli* was carried out at different days (D 0) , D +1, D+3, D + 5 and D + 8.

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II-5- Sensory evaluation:

The evaluation was intended to provide both an idea about the acceptance of the formulations of "Leben" and an overall assessment of the product. The hedonic test was chosen to assess the general level of appreciation samples of flavoured "Leben" by 92 no-trained panellists, with the following conditions: same panel, same place, same coding. In addition, mineral water was made available to the tasters to take your taste buds on their toes after each sample evaluated. The qualification 'subjective' is frequently associated with the sensor technology, as opposed to 'objective' given to instrumental methods, since the taste of an individual varies from one day to another, and it differs from that of its neighbor. **(Lindin and Khramtsov 1991)**

- The criteria for the organoleptic quality of "Leben" were: flavor, taste, texture, consistency, color and odor.

- Terms of evaluation were:

• It takes place in a specific sensory analysis room where lighting and temperature were controlled and hygienic conditions are met (to avoid contamination of our sample).

• To be not quenched, the samples should be numbered (1 to 3).

• Subjects must be insulated to prevent communication between them, which may influence their judgments, so everyone must have his score sheet.

Statistical analysis

All experiments were conducted in triplicate and results are expressed as mean ± standard deviation (SD). The analysis of variance (*ANOVA*) was performed using XLSTAT Release 10 (Addinsoft, Paris, France). Tukey's multiple range test (HSD) was used to compare means of the determined parameters. Evaluations were based on the $p < 0.05$ significance level.

Results and Discussion

III-1- Biological activities of lentisk essential oil:

III-1-1- Antioxidant activities:

Several methods have been developed to measure the efficiency of dietary antioxidants. These methods are based on different kinds of defence systems: scavenging reactive oxygen species (ROS), hydroxyl radicals, reduction of lipid peroxyl radicals, inhibition of the lipid peroxidation and chelating of the metal ions (**Benhammou, Bekkara et al. 2008**)

III-1-1-1- Radical-scavenging test:

Several authors have determined the RSA of plant extracts by measuring the consumption of the DPPH° radical at 517 nm (***(Achat, Tomao et al. 2012)***. This model is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods (**Göktürk Baydar, Özkan et al. 2007**). In this work, the scavenging effect of *P. lentiscus* essential oil was compared with that of BHA **(Fig.05).**

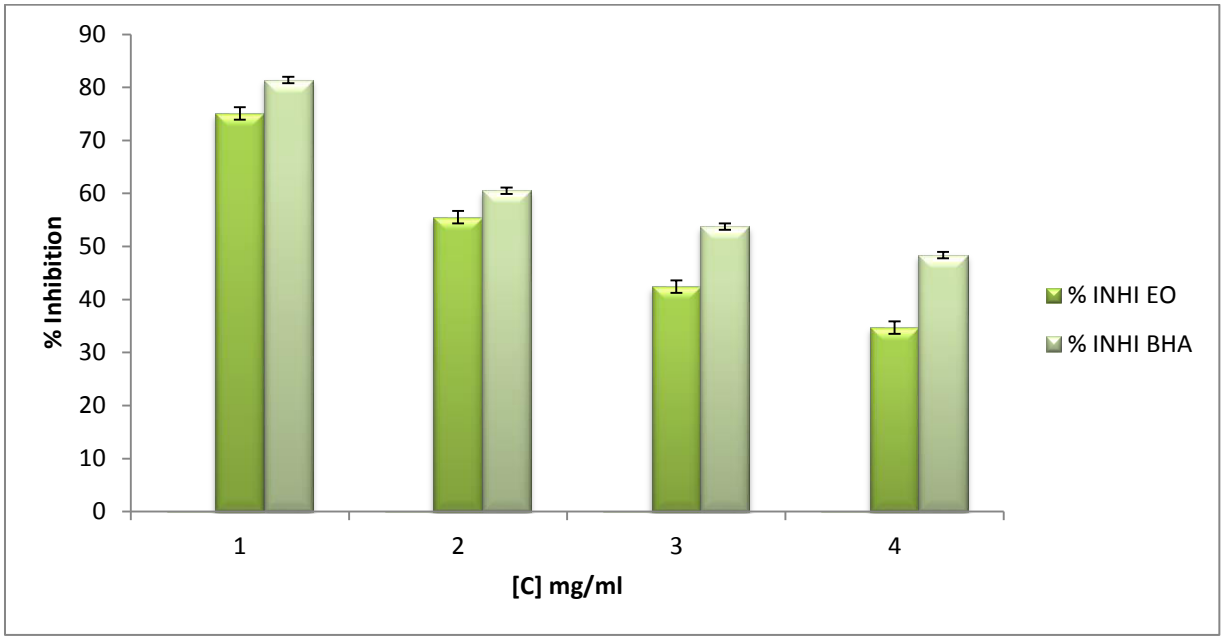

Figure 06: Radical scavenging activities of *Pistacia lentiscus* essential oil and BHA, at different concentrations.

- The results reported in this figure emonstrate the DPPH radical scavenging activities (% inhibition), caused by different concentration of *P. lentiscus* essential oil, which increased with increasing its concentration.

The essential oil exhibited greatest inhibitory activity at concentration of 0.05mg/ml reaching 81.41±0.4. The IC50 values (the concentration reducing 50% of DPPH) obtained for

scavenging activities on DPPH° radical, were evaluated. The lower the IC_{50} value the greater the free radical-scavenging activity. Comparison of the DPPH scavenging activity of the investigated essential oil (21.29±1.03 mg/ml) and those expressed by BHA (8.67 ±0.81 mg/ml) showed that the oil possessed weaker antioxidant effects than BHA.

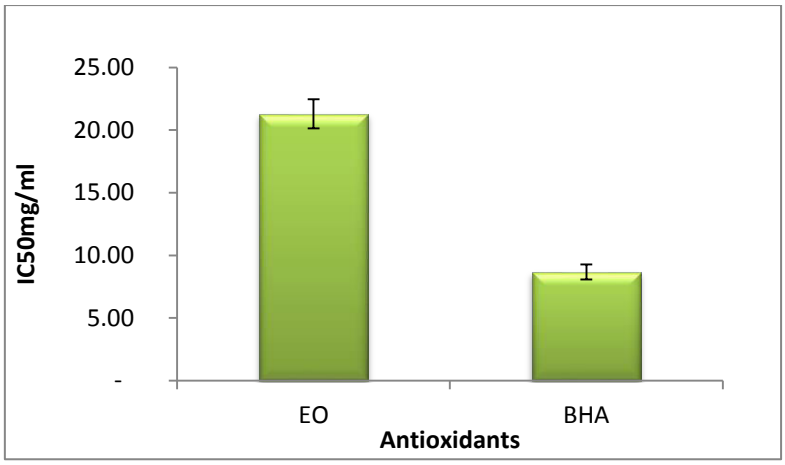

Figure 07: IC_{50} values of *P. lentiscus* essential oil and BHA

This result was higher with that obtained by (**Benhammou, Bekkara et al. 2008**): IC_{50} = 13.5 mg /ml of *P. lentiscus* essential oil. This variation may be due to different growing, climatic and sample extraction methode

III-1-1-2- Reducing power:

Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of antioxidant action, and can be strongly correlated with other antioxidant properties **(Dorman, Peltoketo et al. 2003)**. In this assay, both essential oil and α-tocopherol **(Fig.07),** showed the ability to reduce Fe^{3+} to Fe^{2+}.

The increase in the absorbance at 700 nm of the reaction mixture caused by the *Pistacia lentiscus essential oil* is indicative of their increased reducing power. This activity was somewhat lower than those obtained for the positive control, α-tocopherol at lower concentrations (1 mg/ml)

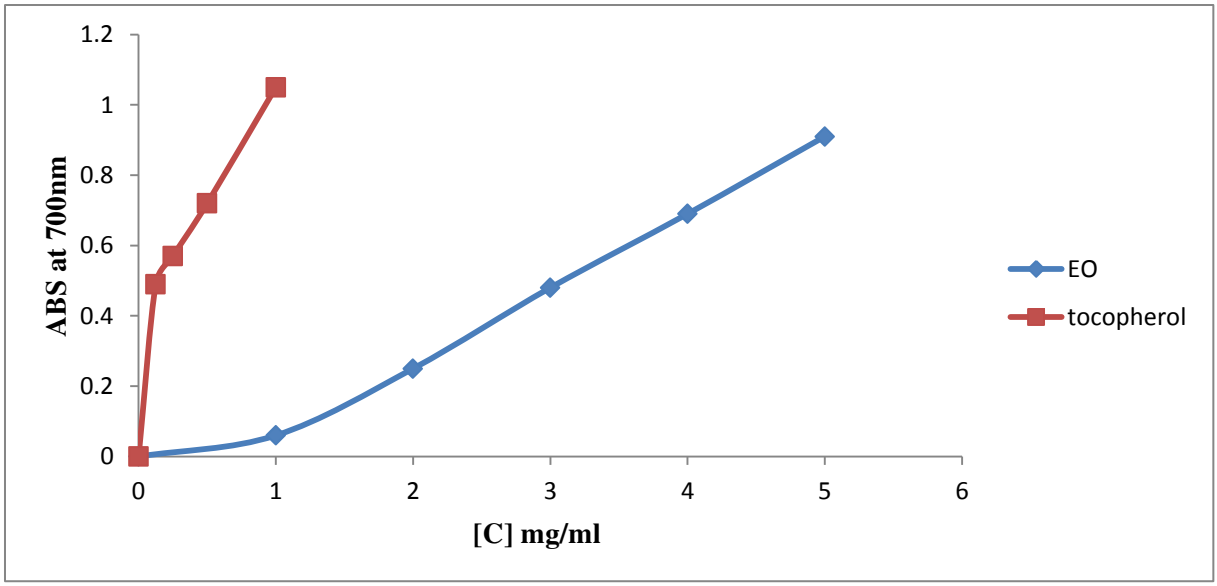

Figure 08: Reducing power of *P.lentiscus* and α-tocopherol in different concentrations.

- RC_{50} was also evaluated (mg ml^{-1}) for lentisk extract and α-tocopherol **(Fig.08)**. This value is the effective concentration at which the absorbance was 0.5, for reducing power and was obtained by interpolation from linear regression analysis. The reducing powers of α-tocopherol maintained the highest levels with of RC_{50} = 0.33 ±0.01.

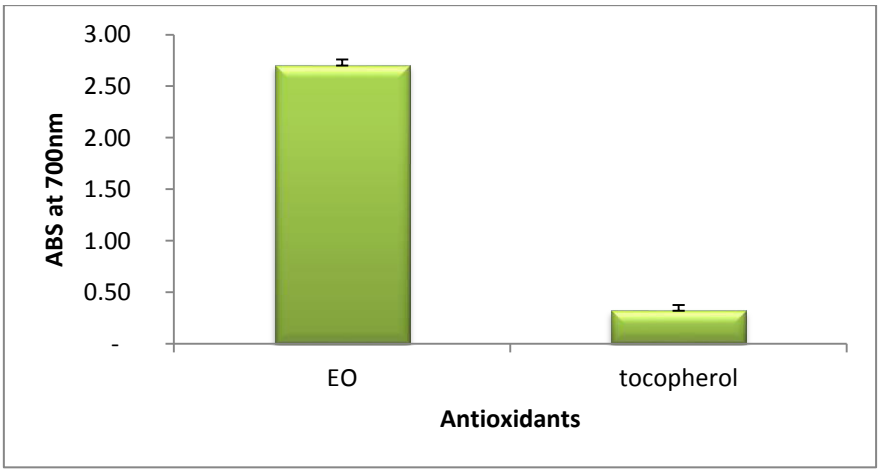

Figure 09: RC_{50} values of *Pistacia lentiscus* essential oil and α-tocopherol in reducing power test.

(Benhammou, Bekkara et al. 2008), found that oil extract from *P. lentiscus* showed high reduction power. At a concentration of 3 mg/mL, an absorbance of 2.862 was recorded.

III-1-1-3- Total Antioxidant activity:

The total antioxidant capacity (TAC) was based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of green phosphate/ Mo(V) complex at acid pH **(Prieto, Pineda et al. 1999).** It evaluates both water-soluble and fat-soluble antioxidants (total antioxidant capacity) **(Jayaprakasha, Girennavar et al. 2008)**. The high absorbance values indicated that the sample possessed significant antioxidant activity. The values of this assay (absorbance at 695 nm) at different essential oil concentrations of *lentisk* were shown in figure 09 and were found to be important and dose dependent but lower than the activity of the synthetic antioxidant BHA.

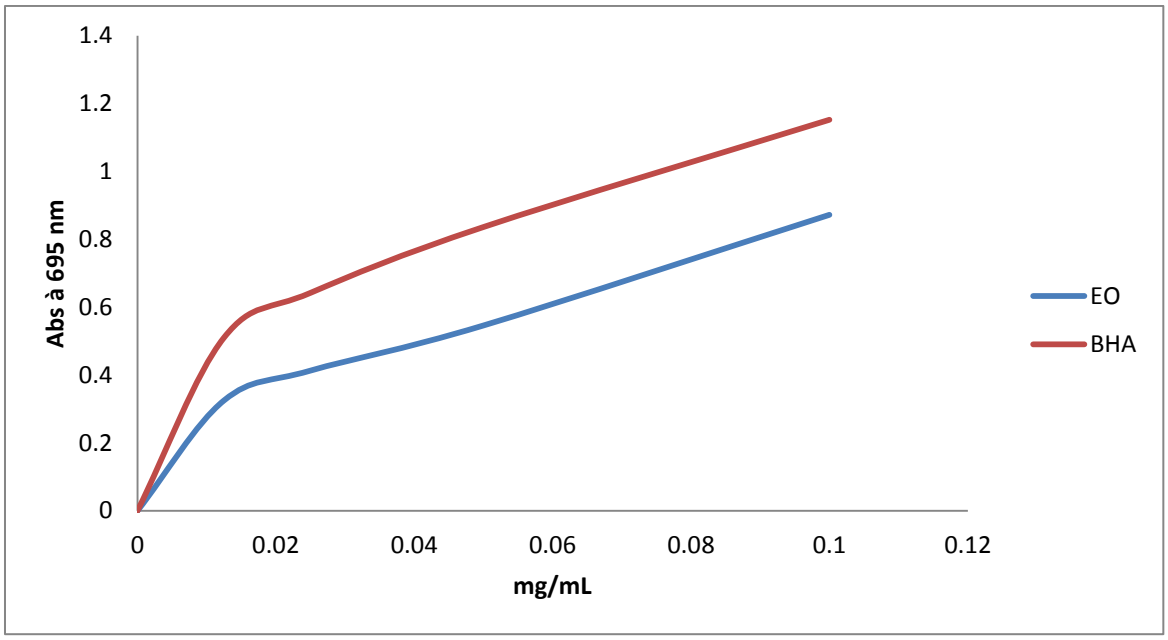

Figure 10: Antioxidant capacity by phosphomolybdenum method of *lentisk* essential oil and BHA

- According to our knowledge, there is not an available literature on total antioxidant activity of *Pistacia lentiscus* essential oil.

III-1-2- Antimicrobial activity:

Antimicrobial activity was assayed against (*S. aureus, E. coli, Yeasts and Moulds*). The essential oil showed a varying degree of antimicrobial activities, these microorganisms have different growth properties. The results of disc diffusion test concentration of essential oils are given in figure 10 and 11.

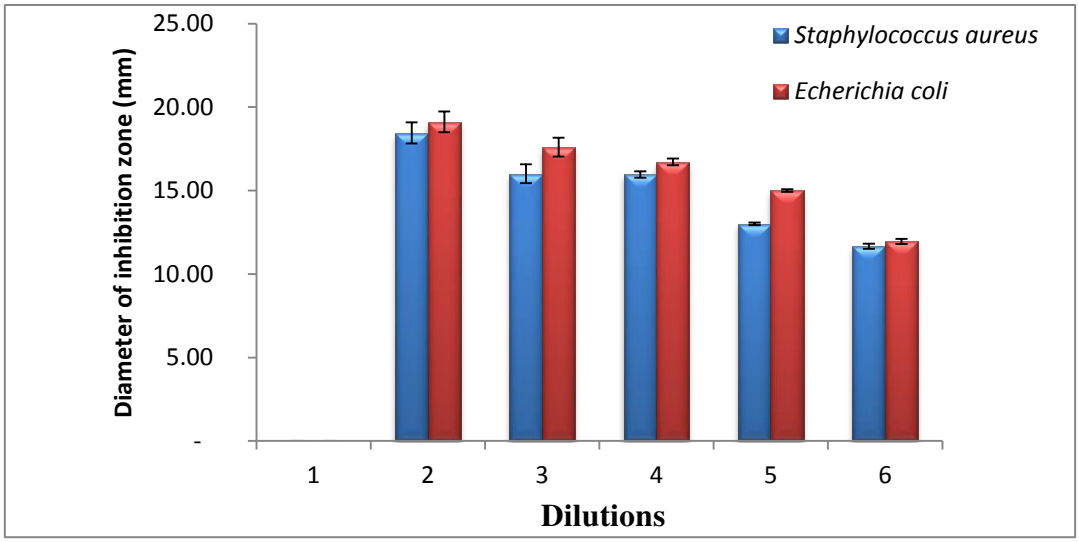

Figure 11: Antibacterial activity of lentisk essential oil.

- Crude essential oil was active against bacterial strains examined while their dilutions induced a decrease in the activity. *E.Coli* is the most sensitive strain tested to the oil of *Pistacia lentiscus* with the strongest inhibition zone of 19.12 ± 06 mm, followed by *S.Aureus* with 18.45±0.07mm. In our study parallel to the study of **(Özçelik, Aslan et al. 2005)** it was found that essential oil mastic obtained from *Pistacia. lentiscus* had serious antibacterial effect on *E.coli, S.aureus* and some other strains. However; the degree of antimicrobial activity is proportional to the essential oil concentration.

- Pinene-type monoterpene hydrocarbons (α-pinene and β-pinene) are well known chemicals having antimicrobial potentials (**Dorman and Deans 2000**). The antibacterial efficacy of essential oil of *Pistacia lentiscus* is probably due to major components; α-pinene **(K. Arab 2014)** and to a number of its components working synergistically **(Derwich, Manar et al. 2010).** Indeed, essential oils rich in α-pinene demonstrated potential antibacterial activity, *Eucalyptus globulus* (**Hajji, Fkih Tetouni et al. 1993**), *Thymus broussonettii* (**Tantaoui-Elaraki, Lattaoui et al. 1993**), and *Mentha rotundifolia (**Derwich, Manar et al. 2010**)*. α-pinene, have been known to exhibit antimicrobial activity against *Escherichia coli and Staphylococcus aureus* **(Moghtader 2009)**. Monoterpenes hydrocarbons, terpinenes, have also shown antimicrobial properties that appear to have strong to moderate antibacterial activity against Gram positive (*Staphylococcus aureus)* and Gam negative bacteria (*Escherichia coli).* **(Oyedeji, Afolayan et al. 2005)**..

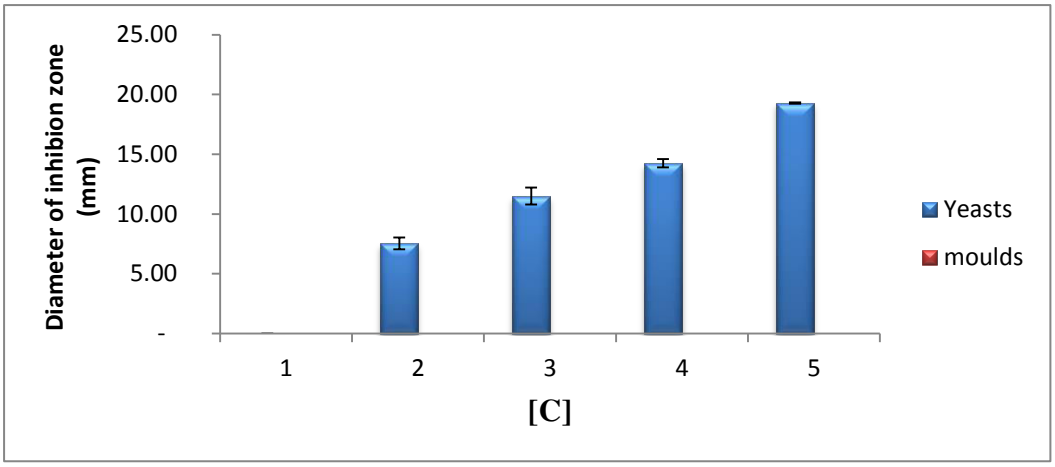

Figure 12: Antifungal activity of *Pistacia lentiscus* essential oil

- The *Pistacia lentiscus* essential oil showed a varying sensibility against yeasts, the greater the volume increase (20, 30, 40 and 60µl), the diameters of the inhibition zones increase, this may be due to the presence of more active compounds with increased volume of essential oil, but did not revealed activity against moulds strain tested.

The study carried out by **(Barra, Coroneo et al. 2007)**, was found that the essential oil of lentisk with the major coumpounds α-terpineol, terpinen-4-ol, α-phellandrene, terpinolene, γ-terpinene,and α-terpinene has no activity against *Fusarium oxysporum*, *Rhizoctonia solani*, *Aspergillus flavus*, and *Pennicillium.* Also, it has been reported **(Kordali, Cakir et al. 2003)** that *Pistacia lentiscus* were examined for their antifungal activities. This extract inhibited the growth of *Pythium ultimum* and *Rhizoctania sambucinum*, but no antifungal activity was observed against *Fusarium solani*. However, this activity depends on the concentration of the oil and mould type.

Monoterpenes such as α -pinene and limonene are among the major components that contribute to strong anti-microbial activity of *Myrtus* (**Rasooli, Moosavi et al. 2010**).

- According to **(Derwich, Manar et al. 2010)**, the antimicrobial activity of monoterpenes is explained by:

- the presence of phenolic hydroxyl groups capable of forming hydrogen bonds with the active sites of the enzymes of the targeted cell;
- the toxic effects on membrane structure and function **(Cox, Mann et al. 2000)**.

- (Sikkema, De Bont et al. 1995) reported that, as a result of their lipophilic character, cyclic monoterpenes will preferentially partition from an aqueous phase into membrane structures.

This resulted in membrane expansion, increased membrane fluidity and inhibition of a membrane-embedded enzyme. In yeast cells and isolated mitochondria, α-pinene and β-pinene destroy cellular integrity, inhibit respiration and ion transport processes and increase membrane permeability**. (Cox, Mann et al. 2000)**

III-2- Analysis of prepared flavored "Leben":

III-2-1- Physico-chemical properties of "Leben"

Physico-chemical properties of the manufactured "Leben" (Standard and flavored "Leben" with two concentration of lentisk essential oil (C_1, C_2), were shown in table 07.

Table 07: Physicochemical analysis of manufactured "Leben"

	pH	**Total dry extract (%)**	**Fat content (%)**
Standard "Leben"	4.55	10.2	3
Flavored"Leben" [C_1]	4.53	10.3	3
Flavored"Leben" [C_2]	4.55	10.1	3

- Results of this analysis revealed that pH, soluble solids content, acidity, fat content determination were conform to norms. Therefore, the *pistacia lentiscus* essential oil has no effect on the physico-chemical of "leben" propreties.

III-2-2- Microbiological analysis of "Leben":

Microbiological quality of the manufactured standard and flavored "Leben", was given in table 08

Table 08: Results of microbiological analysis of manufactured "Leben"

	Total Flora at 37°C	***Staphylococcus aureus***	**Fecal coliforms**	**Salmonella**	**Yeasts & moulds**
Standard "Leben"	*NonC*	Absent	Absent	Absent	Absent
Flavored"Leben" [C_1]	NonC	Absent	Absent	Absent	Absent
Flavored"Leben" [C_2]	NonC	Absent	Absent	Absent	Absent
Norms		**3×10^2**	**30**	**Absent**	

NonC: Non contable = Uncontable.

- Results of this analysis revealed that, microbiological analyses were conform to norms, this illustrates the adequate heating treatment of milk, under strict aseptic conditions, during

processing and manufacturing. The results also indicated that *Pistacia lentiscus* essential oil had no effect on the microbiological quality of the formulated "Leben".

III-3- Incorporation of lentisk essential oil in "Leben": Impact on physicochemicals and microbiolgicals parameters:

III-3-1- Effect of essential oil on pH and acidity of "Leben":

After addition of different concentration of *P.lentiscus* essential oil to the prepared "Leben" , the data, depicted in figures 12 and 13, were recorded. The pH of the standard "Leben" and the flavored "Leben" with different essential oil's concentration decreased slightly over time, whereas the Dornic acidity of the two formulated "Leben" (standard and flavored) increased during storage (30 days).

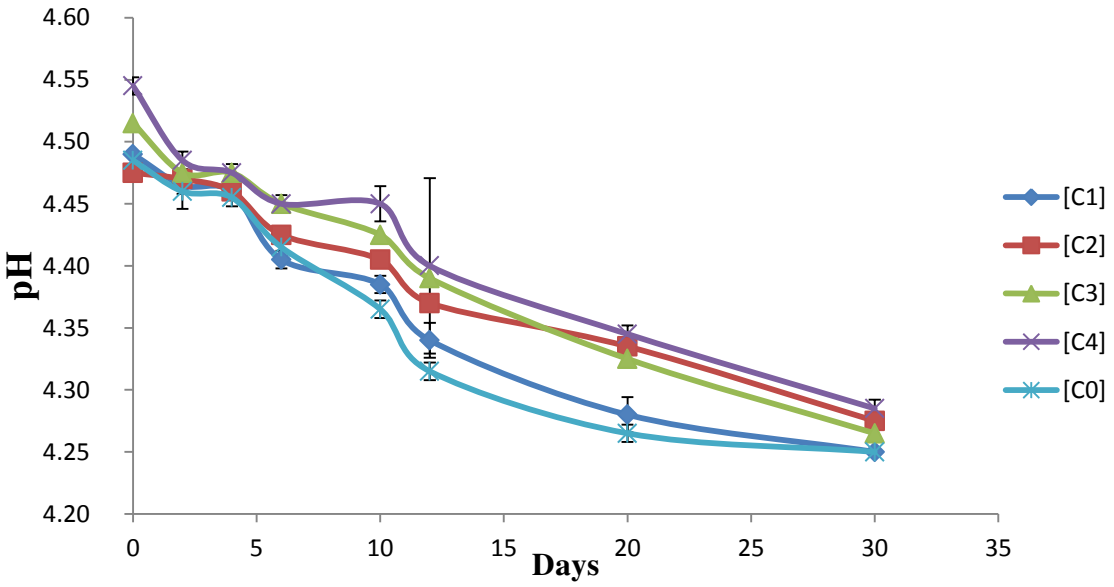

Figure 13: Evolution of pH of standard "Leben" (C0) and flavored "Leben" at different concentrations during 30 days.

- The pH and acidity evolve a manner inversely proportional. But, the more concentrated "Leben" is less acidified through the time, the pH decreased and the titratible acidity increased slightly compared with the standard "Leben". It can be seen that the lentisk essential oil had an effect against the acidification of "Leben" during storage at 4 ° C, for one month. The temperature has not completely stopped the activity of the lactic flora, but just slowed, which could explain the slight acidification.

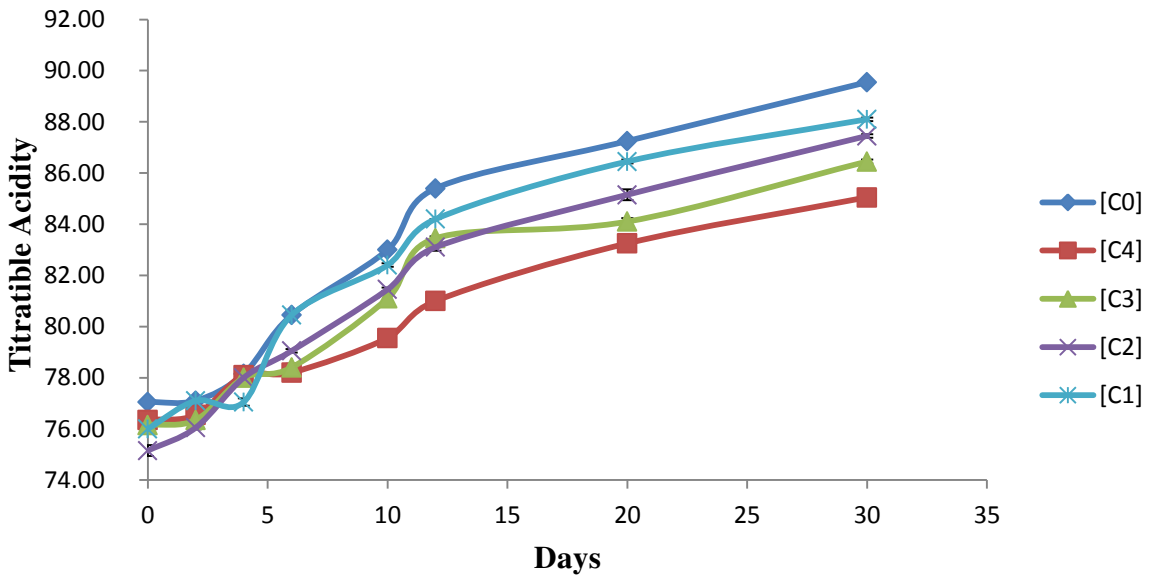

Figure 14: Evolution in titratable acidity of standard "Leben" (C0) and flavored "Leben" at different concentrations during 30 days.

III-3-2- Antibacterial effect of essential oil on contaminated "Leben":

The enumeration of viable microorganisms in contaminated "Leben", was performed after 24h for *E.coli* and 48h for *S. aureus*. The results of this study are shown in tables 09 and 10.

At day 0 (D0), the viable number of the tested strains were uncountable at different concentrations of the essential oil, however at 10% of [C1] and [C2] the effect was immediate. The load decreases in function of time and concentrations. At day 8 (D 8), flavored "Leben" [C2], inhibited completely the growth of the two bacteria.

-Lentisk essential oil exerts considerable activity against the two tested strains and *E. coli*. was inhabitually, more sensitive. This result was not consistent with previous studies reporting that Gram-negative bacteria are more resistant to antimicrobials than Gram-positive microorganisms due to their outer lipopolysaccharide membrane **(Khan, Islam et al. 2009).** Lightly rating decrease load in the controls may be due to the acidity caused by the lactic acid bacteria.

- Considering the large number of different groups of chemical compounds present in EOs, it is most likely that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell. Indeed, the locations or mechanisms in the bacterial cell thought to be sites of action for EO components. An important characteristic of EOs and

their components is their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable. Gram-negative organisms are less susceptible to the action of antibacterials is perhaps to be expected, since they possess an outer membrane surrounding the cell wall which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering. However, not all studies on EOs have concluded that gram-positives are more susceptible **(Burt 2004).**

Table 09: Lentisk essential oil effect on *E.Coli*

	Echerichia coli **(Log N)**									
	[C1] Essential oil					[C2] Essential oil				
	0	0.01%	0.1%	1%	10%	0	0.01%	0.1%	1%	10%
D-0	NonC	NonC	NonC	NonC	ind	NonC	NonC	NonC	NonC	2.90±0.09
D+1	NonC	NonC	NonC	NonC	2.86±0.06	NonC	NonC	NonC	NonC	2.79±0.03
D+3	NonC	2.82±02	2.43±0.05	2.23± 0.09	2.10±0.09	NonC	1.95±0.06	1.95±0.3	1.74±0.2	1.6± 1.26
D+5	3.11±1.3	1.85±0.05	1.78± 0.08	1.6±0.2	0	3.13±1.5	1.38±0.12	1± 0.7	0	0
D+8	2.91±2	1.38±0.12	0	0	0	2.86 ±0.7	0	0	0	0

D: Day, NonC: Non Contable

Table 10: Lentisk essential oil effect on *S.aureus*

	Staphylococcus aureus **(Log N)**									
	[C1] Essential oil					[C2] Essential oil				
	0	0.01%	0.1%	1%	10%	0	0.01%	0.1%	1%	10%
D-0	NonC	NonC	NonC	NonC	NonC	NonC	NonC	NonC	NonC	3.07± 1.7
D+1	NonC	NonC	NonC	NonC	3.19± 0.9	NonC	NonC	NonC	NonC	3.07± 1.7
D+3	NonC	2.96±1.64	2.86± 0.78	2.74± 0.83	2.63± 1.06	NonC	2.79± 2.2	2.75± 0.9	2.61± 0.03	2.54± 2.1
D+5	3.43 ±1.7	1.98±0.09	1.89±0.02	1.80 0.08	0	3.15 ±2.03	1.90± 0.06	1.83 0.9	1.7± 0.3	0
D+8	3.38 ± 1.4	1.60 1.43	1.56±1.06	0	0	2.92 ± 0.8	0	0	0	0

D: Day, NonC: Non Contable

III-4- Sensory analysis:

Three samples of "Leben" (1, 2, 3: flavored [C1], reference and flavored [C2] respectively), were sensory evaluated, and scores were recorded

- Design of experiment

. Designing an experiment is a fundamental step in order to verify if the collected data will be statistically valid (**Périnel and Pagès 2004**). In our study, an optimal plan was validate.

Design evaluation

A-Efficacity	1,000
D-Efficacity	1,000

- Product Characterization

The figure 13 represents the characteristics ordered from the one having the highest discriminating power to the one that has the lowest discriminating power on the prepared leben. As reported, the color has the lowest discriminating power, followed by texture and finally the acidity.

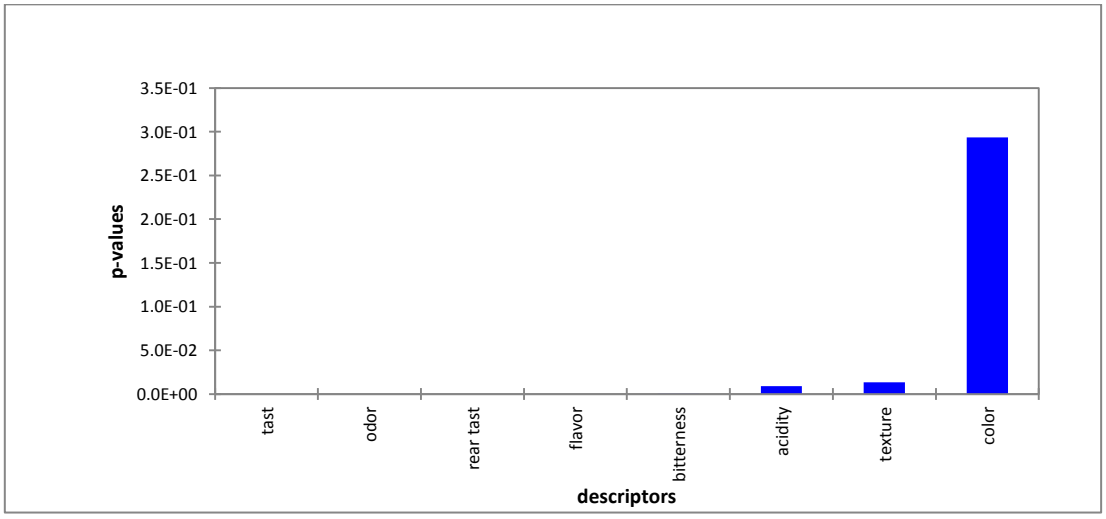

Figure 15: Discriminating power by descriptor

- Model coefficients

This test is intended to treat each combination product-descriptor, the coefficient, the estimated mean, the *p*-value and a confidence interval on the coefficient (**Næs and Risvik 1996**).

- . Blue: the features whose coefficient are significantly positive;
- White: ones whose coefficient is not significant;
- Red: the characteristic that the coefficient is significantly negative.

- The analysis of each graph defines each product **(Fig.16)**

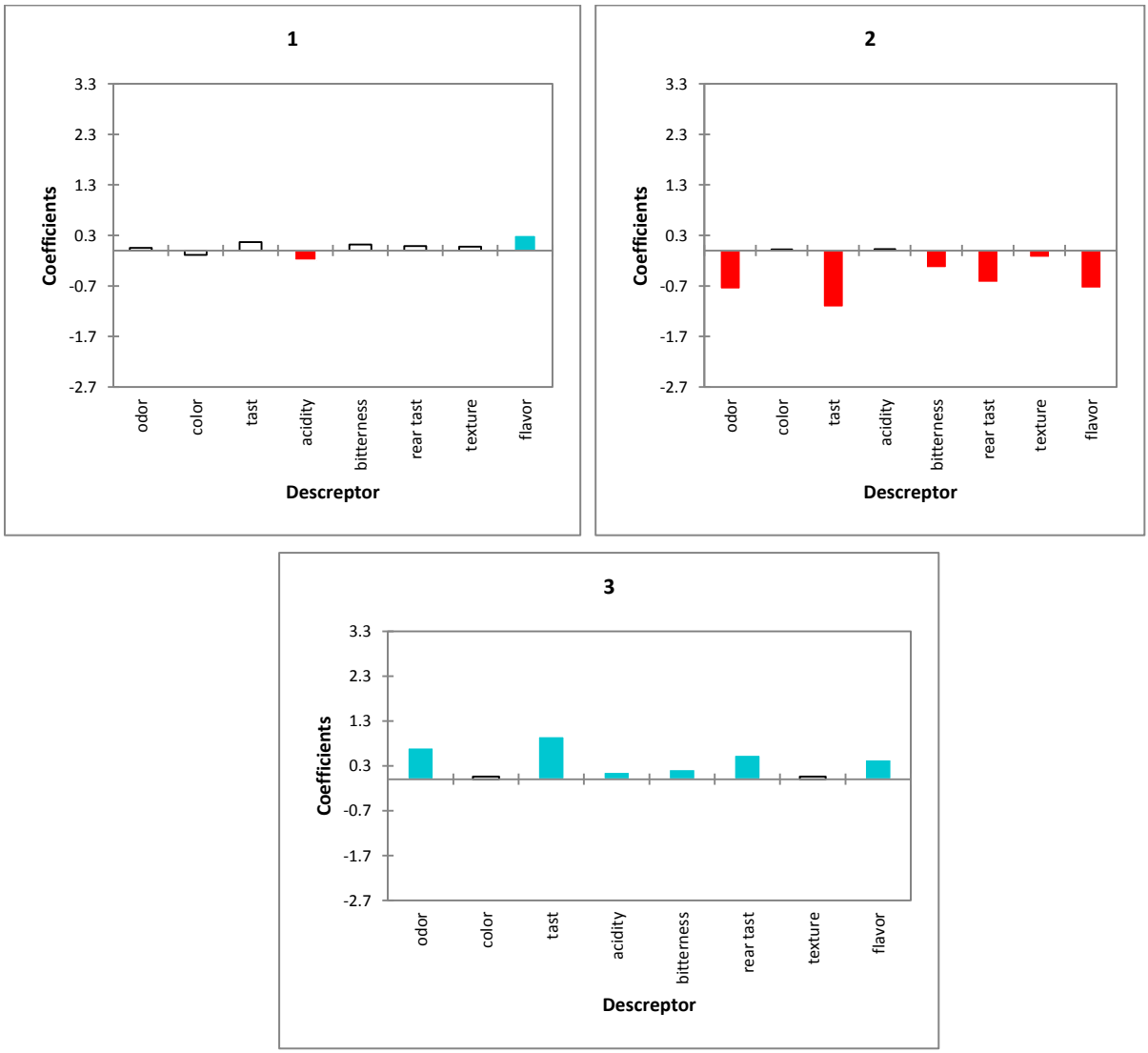

Figure16: Model coefficients of "Leben"

This figure indicated that:

- Sample 1 had a good odor and good flavor but it had a bad acidity.
- Sample 2 had a bad odor, tast, bitterness, texture and flavor
- Sample 3 had a good odor, tast, acidity, bitterness, rear tast, flavor

- Adjusted product

The purpose of this action is to define the adjusted calculated from the model for each combination product-descriptor (Le and Husson 2008). In this sensory evaluation results of table 11 were obtained

Table 11: Corresponds to the adjusted averages calculated from the model for each combination product-descriptor

	bitterness	tast	rear tast	flavor	odor	texture	acidity	color
3	2,381	4,369	3,048	2,845	3,917	2,131	3,238	1,571
1	2,274	3,583	2,595	2,714	3,262	2,143	2,881	1,429
2	1,810	2,286	1,869	1,655	2,440	1,929	3,107	1,536

This table brings out the averages when passing the various Products and features. It is therefore seen in blue averages that are significantly larger than the overall average and red one significantly smaller than the overall average. Mixture 3 had a good tast, rear tast, odor acidity and bitterness as opposed to the sample 2.

- **Preference**

The figure 15 represents the histograms of preference of each product by subjects. As it can be seen, the sample 3: "Leben" flavored with lentisk [C_2] was more appreciated than 1 flavored with lentisk [C_1] and standard "Leben".

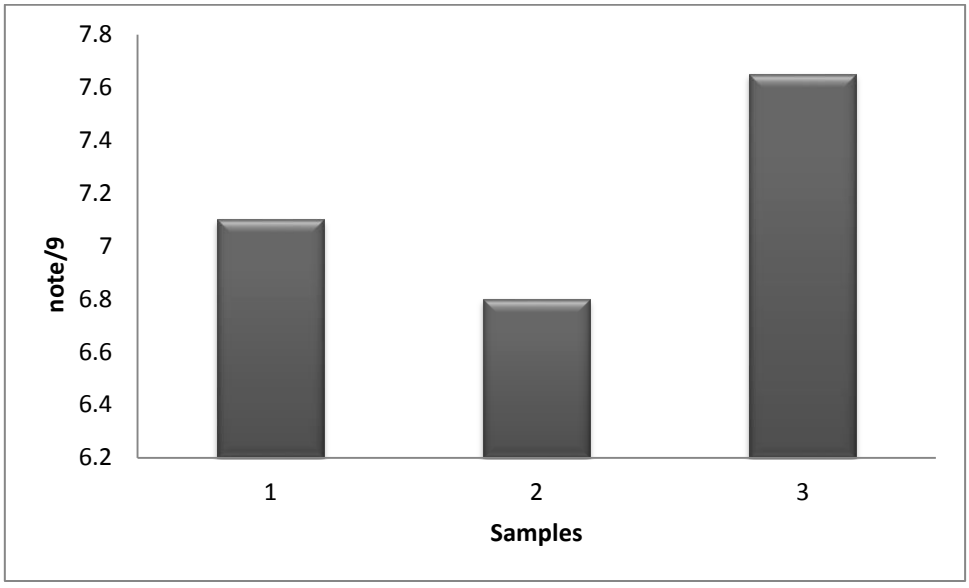

Figure 17: Preferences assigned to each product by subjects.

Conclusion

Conclusion:

According to the results, it may be concluded that *Pistacia lentiscus* essential oil compounds revealed a considerable antimicrobial and antioxidant activity:

- The tested essential oil showed an important scavenging activity for DPPH° radical with IC_{50} = 21.29 ± 1.17 mg/ml (BHA IC_{50}= 8.67 ± 0.6), a reducing power activity with RC_{50} = 2.71 ± 0.05 mg/mL (α-tocopherol RC_{50} = 0.33 ± 0.01 mg/mL), and a total antioxidant activity with A = 0.872 (BHA A = 1.152 at 0.1mg/ml. Therefore this extract possesses antioxidant properties and could serve as free radical inhibitors or scavenger or, acting possibly as primary antioxidants.

The gram negative (*Escherichia coli*), the gram-positive (*Staphylococcus aureus*) and *yeasts* strains tested, were found to be sensitive to the essential oil, that showed a very effective activity with the strongest inhibition zone 19.12 ± 0,08 mm for *E. coli*, followed by *S. Aureus* with 18.4 5± 0.07mm. and 19.29±0.06 mm for yeasts at 60µl of EO. However, this essential oil didn't show any effect against moulds, this is may be due to the chemical composition. It can be concluded that the essential oil of *Pistacia lentiscus* can be used as a good antibacterial agent.

All the physico-chemical and microbiological analysis of the elaborated "Leben" are conform to the norm.

The effect of the incorporation of lentisk essential oil in "Leben" on the physicochemicals parameters, pH and the titratible acidity, indicate an eventual preservation, illustrated by reducing the titrable acidity with the increasing concentration of essential oil.

- Antibacterial activity of essential oil on contaminated "Leben" revealed an interesting effect against *E.coli* and *S. aureus*; at day 0 (D0), the viable number of microorganism were uncountable at different concentrations of the lentisk extract, however at 10% of [C1] and [C2] the effect was immediate and at day 8 (D 8), flavored "Leben" [C2], inhibited completely the growth of the two bacteria.

The sensory analysis revealed that the manufactured "Leben" (03) flavored with *Pistacia lentiscus* essential oil [C2] is the most appreciate by the subjects.

In view of their organoleptic properties, essential oils could most readily be incorporated in manufactured foods that are traditionally associated with herbs (savoury dishes such as meat and fish dishes, cheese, vegetable dishes, soups and sauces) or with spices (drinks and desserts containing fruit and/or dairy products). It may be possible to use EOs in foods not previously associated with a herby or spicy flavour if the presence of one or more

synergists can produce the desired antibacterial effect at a concentration which does not produce undesirable changes in the flavour or aroma. Thus, it is suggested that this plant may be recommended as useful sources to prepare natural bioactive products, with natural preservation agents to help reduce food spoilage.

Therefore, further work should be performed:

- Assessment on the antioxidant activities of formulated “Leben”
- Evaluate the pharmacological activity of this essential oil by screening of such various natural organic compounds and identification of active agents must be considered as a fruitful approach.

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Appandix

Questionnaire d'évaluation hédonique de trois échantillons de Lben

Age : ☐ **Date :**

Sexe : Féminin ☐ Masculin ☐

Trois échantillons de Lben codés 1, 2 et 3 vous sont présentés, il vous est demandé d'évaluer différentes caractéristiques et attribuer une appréciation.

NB : Veuillez rincer votre bouche à chaque dégustation d'un échantillon.

1-Odeur :

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

Echantillon 1	Echantillon 2	Echantillon 3

2-Couleur :

1- Blanc.
2- Beige clair.
3- Beige.
4- Jaune clair.
5- Jaune.

Echantillon 1	Echantillon 2	Echantillon 3

3-Saveur:

a-Gout aromatisé :

1- Très fort.
2- Fort.
3- Moyen.
4- Faible.
5- Absent.

Echantillon 1	Echantillon 2	Echantillon 3

b- Acidité :

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

Echantillon 1	Echantillon 2	Echantillon 3

c-Amertume :

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

Echantillon 1	Echantillon 2	Echantillon 3

d-Arrière gout :

1- Très fort.
2- Fort.
3- Moyen.
4- Faible.
5- Absent.

Echantillon 1	Echantillon 2	Echantillon 3

e-Texture en bouche :

1-Très lisse.
2-Lisse.
3-Moyenne.
4-Granuleuse.
5-Très granuleuse.

Echantillon 1	Echantillon 2	Echantillon 3

f-Arome identifié (Parfum) :

1-La menthe نعناع

2-Céleri كرافز

3-Lentisque amadaɣ الضرو

4-Thym زعتر

5-Non identifié

Echantillon 1	Echantillon 2	Echantillon 3

4- Préférence :

Veuillez indiquer dans le tableau ci-dessous votre préférence selon la note correspondante à son appréciation:

Taux de satisfaction	Produit 1	Produit 2	Produit 3
(1) Extrêmement désagréable			
(2) Très désagréable			
(3) Désagréable			
(4) Assez désagréable			
(5) Ni agréable ni désagréable			
(6) Assez agréable			
(7) Agréable			
(8) Très agréable			
(9) Extrêmement agréable			

5-Quels sont les caractéristiques qui ont motivé votre préférence ?

1- Odeur.

2-Couleur.

3-Gout aromatisé.

4-Texture en bouche.

5-L'ensemble des caractéristiques évalués.

Echantillon 1	Echantillon 2	Echantillon 3

Merci pour votre participation

Abstract

Essential oils was exploited as the natural additives in fermented milk. The objectives of this study are the evaluation of mastic leaves through the use of its essential oil as a preservative and natural aromatic agent in" leben". the results of the antioxidant activity of this essential oil are: for the DPPH the EC50 is 21.29±1.03 mg/ml , for the reducing power RC0.5 is 0.71± 0.05, for total Antioxidant activity the Abs is 0.872 at 0.1mg/ml. indeed the antimicrobial activity of *Pistacia lentiscus* against *staphylococcus aureus, Echerichia coli* and yeasts and molds were evaluated using standard agar-disk diffusion assay . E.Coli is the most sensitive strain tested to the oil of *Pistacia lentiscus* with the strongest inhibition zone of 19.12mm, followed by *S.Aureus* with 18.45mm.and showed a sensibility against yeasts but did not show activity against all moulds strain tested. The studying of the effect of pistacia lentiscus essential oil on the pH and the titratible acidity indicate that has an effect of preservation, illustrated by reducing the titrable acidity with increasing on the concentration of essential oil. For the subjects preferences in sensory analysis, the flavored leben obtained with essential oil [C2] is the most appreciate.

Keywords: *Pistacia lentiscus L.*, essential oil, antioxidant activity, antimicrobial activity, fermented milk, preservative, sensory analysis.

Résumé

Les huiles essentielles ont été exploitées comme additifs naturels dans le lait fermenté. Les objectifs de cette étude sont l'évaluation de l'activité des feuilles du lentisque par l'utilisation de son huile essentielle comme agent de conservation et de l'agent aromatique naturel sur "leben". les résultats de l'activité antioxydante de cette huile essentielle sont: pour le DPPH la CE50 est 21,29 ± 1,03 mg / ml, pour la puissance RC0.5 réducteur est de 0,71 ± 0,05, pour l'activité antioxydante totale l'ABS est 0,872 à 0,1 mg /ml. En effet, l'activité antimicrobienne du lentisque contre *Staphylococcus aureus*, *Escherichia coli* et des levures et moisissures ont été évaluées en utilisant la methode de diffusion des disque sur gélose. *E.Coli* est la souche la plus sensible testée à l'huile essentielle du lentisque avec la zone d'inhibition plus grande de 19.12mm, suivie par *S.aureus* avec 18.45 mm. Elle a aussi montré une acivté contre les levures, mais n'a pas montré une activité contre les moisissures L'étude de l'effet de l'huile essentielle *Pistacia lentiscus* contre le pH et de l'acidité titrable a indiqué qu'elle a un effet de préservation, illustrée par la réduction de l'acidité titrable avec l'augmentation de la concentration de l'huile essentielle. Pour les sujets dans l'analyse sensorielle, a indiqué la préférence pour le produit (03) aromatisés avec la concentration [C2].

Mots clés : *Pistacia lentiscus L*, Huile essentielle, Activité antioxydante, Activité antimicrobienne, Lait fermenté, conservateurs, Analyse sensorielle.