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Essential oil composition of *Origanum vulgare* and its application in substitution of synthetic chemical additives

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ABSTRACT

In this work, the Gas Chromatography-Mass Spectrometry (GC/MS) analysis of the essential oil of *Origanum vulgare* L. obtained by hydrodistillation was carried out and it allowed to identify 98.1% of its constituents. The main components were carvacrol (47.6%), thymol (16.6%), *p*-cymene (13.5%) and γ -terpinene (11.2%). The chemical components of the essence are distributed over five biochemical classes represented mainly by monoterpene phenols and monoterpene carbides. The extraction gave a yield of oil 2.8%. The minimum inhibitory concentration (MIC) of this oil was determined against food spoilage bacteria and pathogenic bacteria belonging to the genus *Staphylococcus* tested *in vitro*. This study aims to enhance the value of oregano essential oil by determining its chemical composition, and by its application as a natural antibacterial preservative in substitution of synthetic chemical additives. The sample was incorporated into a sensitive food matrix exposed to bacterial contamination: white meat. The bacteriostatic/bactericidal power of this bioactive extract was determined by a significant reduction in the number of bacteria and a clear increase in shelf life. Thus, it is possible to propose the use of this aromatic essence as a source of natural preservative.

Keywords: *Origanum vulgare*, essential oil, analytical study, white meat, microbiological analysis, preservative effect.

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INTRODUCTION

One of the main problems of the food industry is to ensure that food products are well preserved, particularly against microbial contamination, which is the most feared. The latter can be caused by various microorganisms such as viruses, yeast parasites, moulds and bacteria. There are two types of contaminating bacteria; spoilage bacteria, that are harmless to the consumer but cause various food spoilage; and potentially pathogenic bacteria, which can cause infection or toxic infections when they produce enterotoxins (Le Loir *et al.*, 2003; Jenny *et al.*, 2011) and even lead to serious infections. The presence of these bacteria in food lead to a reduction in nutritional values through loss of vitamins and modification of organoleptic properties through their proteolytic and lipolytic activity and produce molecules with unpleasant odours through deamination of amino acids, decarboxylation of amino acids and finally degradation of sulphur amino acids, and sometimes

even the appearance of toxic substances (Pascal, 1979; Nessrien & Mohamed, 2007).

Meat is undoubtedly one of the foods most exposed to this scourge. For nutritional reasons, this foodstuff occupies a prime position in the human diet (Clinquart *et al.*, 1999). Its composition in water and proteins of high biological value makes it a very favourable niche for the development of microorganisms that can reach a dangerous threshold (Benaissa, 2014). Therefore, it is the object of increasing concern in modern society. In the past, it was preserved by drying, salting or smoking (Collin, 1972), but to avoid the problem of contamination, it is common practice in the food industry to add synthetic chemicals (Patrone *et al.*, 2010). Whether or not they have a nutritional value and whose intentional addition has a technological or organoleptic purpose, they can become a component of the foodstuff, such as food additives. Synthetic additives include antibacterial preservatives (Nakahara *et al.*, 2003).

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The issue of greatest concern for public health remains the impact of these compounds on the consumer (Burt, 2004) due to their short- and long-term toxic, neurotoxic, mutagenic and even teratogenic effects (Ho *et al.*, 2009; Chahardehi *et al.*, 2010). Several synthetic preservatives have been restricted or banned in several countries. Antibiotics, among them, occupy a significant place as preservatives against food spoilage. Developing countries are the most sensitive to this problem, suffering enormous damage, leading to immeasurable economic losses and major health risks (Ownagh *et al.*, 2010). This has prompted the search for alternative solutions to combat resistant bacteria, through the search for new antibacterials such as bacteriophages, peptides and plants.

Currently, the focus is on aromatic and medicinal plant extracts such as essential oils, which are experiencing renewed interest. Although these essential oils are mostly toxic, many authors (Rota *et al.*, 2008; Kempf *et al.*, 2011; Amrouni *et al.*, 2014; Atilia *et al.*, 2015; Heni *et al.*, 2015a; Heni *et al.*, 2015b) report an antibacterial effect. They contribute to the improvement of taste qualities, and are classified as “Generally Recognized As Safe” (GRAS) and approved for food use by the US Food and Drug Administration (Lis-Balchin, 2004; Caillet *et al.*, 2007; Buronzo, 2008). Among the aromatic and medicinal plants is oregano, which is a spontaneous and abundant plant in north-eastern Algeria. It is part of the Algerian culinary heritage and is used fresh or dried as a spice in certain meat dishes, both for its preservative qualities and its savoury taste. It is also used in the control of microorganisms, which could reduce the risk of spoilage and ensure food safety for consumers. Moreover, this plant is an important source of bioactive molecules (Bruneton, 2008). Among these substances, essential oils with a complex chemical composition and rich in active compounds, act on various cellular targets; this contributes to the resolution of the issue of bacterial resistance.

A chemical screening of the extracted oil was carried out and this study aims to highlight its probable antibacterial activity through its use as a preservative for a sensitive foodstuff of essential nutritional value: white meat. The results of the experiments allowed to calculate the bacterial reduction capacity in this foodstuff and to study its activity over time.

MATERIALS AND METHODS

Plant Material and Essential Oil Extraction

The aerial parts of *Origanum vulgare*, were collected from the region of Hammadi Krouma (Skikda, North-East, Algeria). The plant was identified by Dr. Hicham Boughendjioua at the Department of Natural Sciences, Higher School of Professors for Technological Education, Skikda, Algeria. The voucher specimen (Boughendjioua 01/PPL/2020) under the plant's name was deposited in the herbarium.

The essential oil (EO) of the aerial parts of *Origanum vulgare* L. was obtained by hydrodistillation using a Clevenger type apparatus. An analytical study was carried out to evaluate

the quality and chemical composition of the studied oil, first by determining the physicochemical properties. The identification of the chemical composition was carried out by Gas Chromatography-Mass Spectrometry (GC-MS). This allows the identification of the constituents through their molecular mass, depending on the operating conditions.

The Use of Essential Oil as an Antibacterial Substitute for Food Additives

The effectiveness of the essential oil tested is evaluated by measuring two concentrations: the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC). These concentrations allow to know the nature of the antimicrobial activity of the essential oil: bacteriostatic or bactericidal. In general, essential oils have quite strong and powerful odours, which may be a limiting factor for their application in foodstuffs. However, undesirable organoleptic effects can be limited by careful selection of the EO according to the type of food being considered (Burt, 2004). On the other hand, the values of the minimum inhibitory concentrations (MICs) obtained *in vitro* should be affected by a correction coefficient ranging from 2 to 100, so that they have the same effect in a food matrix (Burt, 2004). For this reason, it is necessary to determine the MIC of the EO, capable of inhibiting bacterial growth without altering the organoleptic characteristics of the food, namely taste and odour.

Analysis

Sensory analysis

The sensory analysis was carried out with a panel of ten people on white meat with the extract tested added. The scoring tests were carried out on taste and smell after cooking. After the taste test was performed and the taste was found to be good and the smell unnoticeable, we were able to determine the MIC of EO added to the meat. This is why prior tasting tests in this kind of work are indispensable. Therefore, we found it useful to multiply the MIC value by a correction factor of 4.

Microbiological Analysis of Test Samples

White meat samples (chicken steak) were spiked with 0.1% *Origanum vulgare* EO and stored at 4°C for 48 h. To determine the bacterial burden at different storage times (0 min- 40 min- 24 h and 48 h), the same protocol was followed for control samples (no EO added) under the same conditions.

Quantitative Analysis

Contamination microflora (total aerobic mesophilic flora) was studied by the Colorimetry method in liquid medium (most probable number, MPN), for both samples, with EO and the control sample; during the different storage times from 0 min to 48 h. The inoculation was carried out from dilutions (10^{-1} , 10^{-2} and 10^{-3}) and we introduced 1ml of the inoculum into

tubes containing 7ml of nutrient broth (a series of 4 tubes for each dilution). The tubes were well homogenised with a shaker. Incubation of the seeded sets of tubes was done at 37°C for 24 h. Tubes showing cloudiness of the nutrient broth medium were considered positive. The final reading, as well as the bacterial count by the MPN test, was performed according to the Mac Grady table. Each inoculation was performed in triplicate.

Pathogenic microorganisms (*Staphylococcus aureus*) studied by inoculating Petri dishes containing Chapman's selective medium with 0.1 ml of the dilution (10^{-1}) on the surface using a raking pipette. The seeded dishes were then incubated at 37°C for 24-48 h. *Staphylococcus aureus* is pigmented yellow, surrounded by a clear halo. Non-pathogenic staphylococci are characterized by transparent, slimy colonies. The number of *Staphylococcus aureus* was determined in Colony-Forming Units (CFU/g) of the sample. Each inoculation was performed in triplicate.

Calculate the Bacterial Reduction Capacity

We calculated the bacterial reduction capacity of the tested extract and thus studied its activity over time. This reduction capacity considered by this study is the difference between the number of bacteria sought in meat without extract and that sought in meat with added oil, for the contamination flora and *S. aureus* enumerated during the different conservation times. The capacity to reduce the bacterial load is expressed as a percentage and is calculated according to the following formula:

$$\text{Reduction capacity} = [(NA - N)/NA] \times 100$$

where, NA is the number of bacteria calculated in the non-added meat and N is the number of bacteria calculated in the added meat.

Statistical Analysis of Results

The results were analysed by calculating the means plus or minus the mean standard deviation. They are compared to the control by Student's t-test (STATISTICA 8). The confidence level is 95.00% and the difference between the means is considered: very highly significant if $p \leq 0.01$, highly significant if p is between 0.01 and 0.1; there is no significant difference if $p \geq 0.5$.

RESULTS AND DISCUSSION

Analytical Study of the Essential oil of *Origanum vulgare*

Table 1. Physico-chemical parameters of *Origanum vulgare* essential oil

Physico-chemical parameters	<i>Origanum vulgare</i>	AFNOR standards
Relative density	0,920	[0,894 – 0,930]
Refractive index	1,4920	[1,4830-1,510]
Miscibility in ethanol	(2v/1v)	[2v/1v/ 3v/1v]
Acid index	4,9	[4,1 – 5,2]
Ester index	51,98	[49,5 – 52,55]

The yield of extracted oil was 2.8% and it is an important source of bioactive molecules. Table 1 shows that the physico-chemical parameters of the essential oil are of a quality that meets AFNOR (2000) standards and is relatively pure.

Chemical Composition

Chemical composition analysis (Figure 1) showed a wide range of compounds. The essential oil is composed of 25 compounds that represent about 98.1% of the total oil (Table 2). The oil consists mainly of Carvacrol (47.6%) followed by Thymol (16.6%), p-cymene (13.5%) and γ -terpinene (11.2%).

The constituents of the essential oil are divided into five biochemical classes represented mainly by monoterpene phenols and monoterpene carbides (Figure 1).

Table 2. Chemical composition of *Origanum vulgare* essential oil

Peak No.	Retention time (Rt)	Compounds	%
01	2.99	α -thujene	0.3
02	5.42	α -pinene	0.7
03	5.64	Camphène	2.1
04	6.02	Octen-3-ol	0.1
05	6.75	3-Octanone	0.2
06	6.94	β -pinene	0.2
07	7.01	β -Myrcène	0.3
08	7.48	α - phellandren	0.2
09	7.79	α - terpinene	1.6
10	8.11	p-cymene	13.5
11	8.18	β -phellandren	0.2
12	8.24	Limonène	0.3
13	8.30	3-p- Menthanone	0.6
14	9.07	γ -terpinene	11.2
15	10.36	Cis- β -Ocimene	0.3
16	10.48	Terpinolène	0.2
17	13.08	Linalool	0.3
18	14.50	Bornéol	0.2
19	16.15	Terpinen-4-ol	0.4
20	17.29	Carvacrol	47.6
21	17.51	Thymol	16.6
22	21.73	α -Terpeneol	0.3
23	22.61	β -Caryophyllene	0.2
24	23.79	β -Bisabolène	0.3
25	24.51	Sesquiphellandrene	0.2
Total			98.1

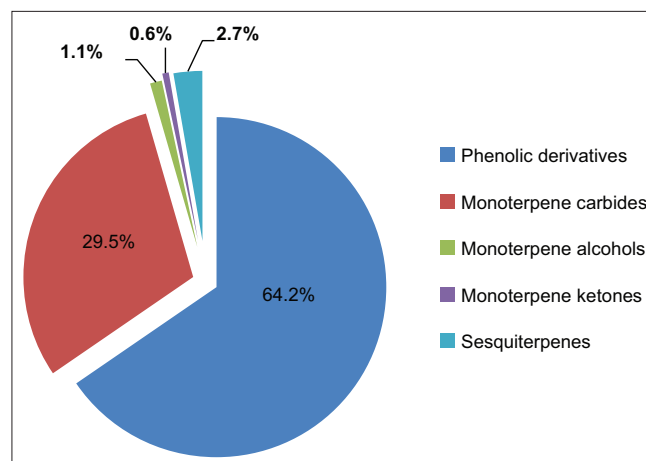


Figure 1: Biochemical classes of *Origanum vulgare* essential oil

Microbiological analysis

Enumeration of total aerobic mesophilic flora and *Staphylococcus aureus*

Quantitative analysis showed that from the first hours of storage, the oil allowed a reduction in bacterial numbers, whereas in the other specimens without EO, there was an increase in the latter. This shows that the effect of the EO is instantaneous, it progresses in time and is maintained up to 48 h of storage at 4°C. The results in the form of averages are grouped in (Table 3). The addition of the essential oil shows an immediate bacteriostatic inhibition, with a slight reduction of the bacterial load of the contaminating flora and of *Staphylococcus aureus*. In fact, the inhibiting effect of the oil is maintained after 40 minutes of conservation at 4°C, with a decrease in the number of bacteria for the total aerobic mesophilic flora and a remarkable decrease on the Chapman medium. This again shows the antimicrobial power of the tested extract. This effect is maintained at an acceptable level despite the storage time and temperature.

Table 3. Variation of the bacterial load at different storage times

Time	Bacterial physiological groups			
	Control sample		Sample added with EO	
	Bacterial load of total aerobic mesophilic flora (CFU/g)	Bacterial load of <i>S. aureus</i> (CFU/g)	Bacterial load of total aerobic mesophilic flora (CFU/g)	Bacterial load of <i>S. aureus</i> (CFU/g)
T ₁	8.2x10 ² ± 0.033	1.1x10 ² ± 0.02	9.9x10 ² ± 0.06	2.1x10 ³ ± 0.08
T ₂	7.5x10 ² ± 0.02	5.8x10 ² ± 0.01	1.13x10 ³ ± 0.04	3.6x10 ³ ± 0.05
T ₃	6.1x10 ² ± 0.002	4.2 x 10 ² ± 0.001	2.5x10 ³ ± 0.01	5.8x10 ³ ± 0.02
T ₄	2.4x10 ² ± 0.001	3x10 ² ± 0.002	3.1x 10 ³ ± 0.02	6.2x10 ³ ± 0.04

The inhibitory effect of the EO was maintained after 24 h of storage as well, and the bacterial count became significant for the sample without the essence and reached its maximum for both bacterial physiological groups. The addition of the additive to the sample resulted in an accentuated reduction of the bacterial load for the total aerobic mesophilic flora and significant for *S. aureus*. For the unsupplemented sample, and after 48 h of conservation under the same conditions, the bacterial load is greater for the total aerobic mesophilic flora and reaches its maximum for *S. aureus*. For the sample enriched with EO, the reduction of the bacterial load is highly significant compared to the control sample, without additive.

Bacterial Load Reduction Capacity

The reduction capacity considered by this study is the difference between the bacterial load of the total aerobic mesophilic flora and *S. aureus* in the sample without essential oil and the one enriched with extract, from the results obtained, it appears that the tested oil showed a reduction rate ranging from 17.17% to 92.25% for the total aerobic mesophilic flora

and much higher for *S. aureus*, 52.38% to 95.18% (Figure 2 and Figure 3). It is evident from our results that the application of *Origanum vulgare* EO at a low concentration that did not alter the organoleptic characteristics of the food matrix (according to the taste test), allowed a reduction of the bacterial load for the different storage times, compared to the control sample.

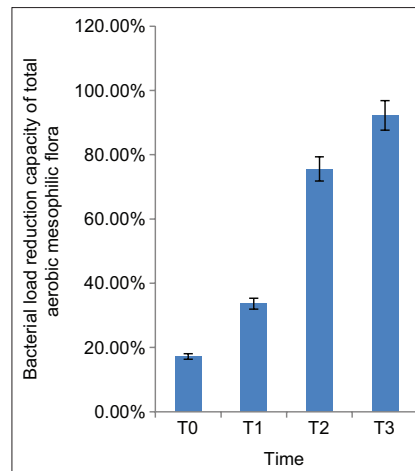


Figure 2: Bacterial load reduction capacity of total aerobic mesophilic flora

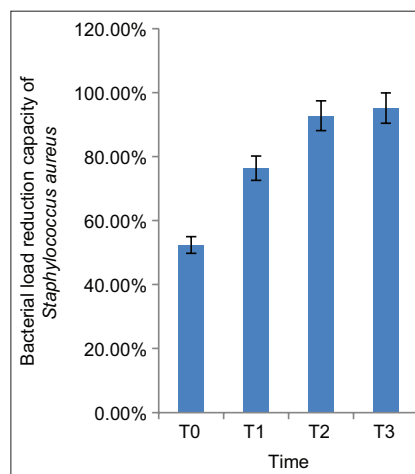


Figure 3: Bacterial load reduction capacity of *Staphylococcus aureus*

It turns out that the inhibitory effect of this bioactive extract is instantaneous, it progresses in time and is maintained until 48h of conservation at 4°C, allowing a significant reduction (0.1<p<0.01) of the sought bacterial load and very highly significant (p≤0.01) of that of *S. aureus*, according to the statistical analysis. From this, in the presence of EO and from 40 min until 48h of conservation, the number of *S. aureus* reached a value almost zero. These results are confirmed by another study conducted on red meat (Heni et al., 2020). This explains that *Oregano* EO has a very strong anti-staphylococcal activity once it is in contact with the target bacteria (bactericidal effect).

The progress of the inhibitory effect of oil could be due to the storage conditions (4°C). However, the availability of nutrients in the meat such as fats, proteins; antioxidants, salt and other substances, as well as pH, temperature, type of packaging

and characteristics of the microorganism, can undoubtedly influence the activity of EO. For example, according to Holley and Patel (2005), at low pH, the hydrophobicity of some EOs increases, allowing them to dissolve easily in the lipid phase of the bacterial membrane.

Burt (2004) suggested that low water content in food may hinder the action of antimicrobial agents towards target sites in the bacterial cell. Thus, the high level of water and salt would facilitate the action of EOs in meat products. By forming a protective layer of fat around the bacteria or the lipid fraction in the food may absorb the antimicrobial agent by decreasing its concentration and efficacy in the water phase. The fact that Oregano EO is among the most suitable for application in meat and meat products (Caillet and Lacroix, 2007) and the specificity of its antibacterial activity. This study affirms the renewed interest in essential oils, which successfully demonstrates their potential use to reduce or control pathogenic flora in food products as an alternative to chemical additives.

It should be noted that our bioactive extract, rich in phenolic derivatives, is very active; a situation reported by Burt in his comparative study based on the content of oils (Burt, 2004). This activity is attributed to Carvacrol and Thymol (Kempf et al., 2011). Thymol, being their major component, binds to membrane proteins and increases permeability, destabilizing cell integrity. It also interferes with the synthesis of structural components (Trombetta et al., 2005) and energy metabolism leading to cell death (Ultee et al., 2002; Nguefack et al., 2004; Cristani et al., 2007). Carvacrol, thus present, accentuates this effect by inhibiting ATPase activity (Gill & Holley, 2006), without neglecting the activity linked to monoterpene hydrocarbons, notably p-cymene and γ -terpinene, which are present in sufficient quantities in our oil. These are precursors of carvacrol biosynthesis. They facilitate its intracellular penetration thus potentiating its action (Ultee et al., 2002).

The progress of the inhibitory effect of oil could be due to the storage conditions (4°C). However, the availability of nutrients in the meat such as fats, proteins; antioxidants, salt and other substances, as well as pH, temperature, type of packaging and characteristics of the microorganism, can undoubtedly influence the activity of EO. For example, according to Holley and Patel (2005), at low pH, the hydrophobicity of some EOs increases, allowing them to dissolve easily in the lipid phase of the bacterial membrane.

In addition, a strong affinity for cytoplasmic membranes can disrupt and affect by causing their swelling to a greater extent than carvacrol (Burt, 2004). The antibacterial activity of essential oils can also be attributed to the phenomenon of synergy between all the volatile constituents; the synergistic interactions between the different compounds can be at the origin of a much more pronounced activity than that predicted for the majority of compounds (Lahlou et al., 2004). The antibacterial activity depends on the chemical composition of the EOs and is related to the bacterial physiological groups.

CONCLUSIONS

The essential oil of *Origanum vulgare* is highly appreciated in terms of taste, and its addition to white meat showed an immediate bacteriostatic inhibiting effect with a lytic power beyond forty minutes. Thus, a highly significant reduction capacity of the total aerobic mesophilic flora and very highly significant of *Staphylococcus aureus* was recorded. This inhibitory effect progresses over time and is maintained up to 48 hours of storage at 4°C. The reduction power is such that it is almost total for staphylococci after 48h. This explains why the essential oil of Oregano has a bactericidal effect on *Staphylococcus aureus*, confirming the results obtained *in vitro*. The present study suggests the use of this type of bioactive molecule in foodstuffs as a substitute for the usual chemical additives, which opens up the prospect of its use in preventing and combating the deterioration of preserved food products. In addition to its economic impact, this oil would contribute to the fight against food poisoning and multi-resistant bacteria.

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