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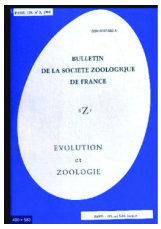

Effect of lemon, thyme and oregano essential oils on organoleptic quality and histamine production in sardine fillets stored at 10°C

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Abstract	The use of essential oils is considered an emerging approach to preserving food quality due to the antibacterial phenolic compounds they contain. The antibacterial power, histamine content and the influence of incorporating lemon, thyme and oregano essential oils on the organoleptic quality of sardine fillets, stored at 10°C was evaluated. The essential oils studied showed significant antibacterial activity against *Klebsiella ozaena*, *Enterobacter cloacae*, *Morganella morganii*, *Proteus mirabilis*, *Citrobacter freundii* and *Providencia rustigiani*. Lemon EO had the lowest inhibition diameter, while thyme EO showed the highest. Application of these oils at a concentration of 5% to sardine fillets revealed a beneficial effect on sensory attributes, improving odor and taste and preserving the organoleptic quality of the sardine up to the sixth day of storage at 10°C without reaching the organoleptic rejection threshold. The essential oils studied controlled bacterial multiplication: thyme EO revealed a significant reduction in bacterial microflora of around 57%, demonstrating bactericidal activity, while the application of oregano EO favored bacteriostatic action, while lemon EO retarded bacterial proliferation. In addition, no histamine production occurred following the incorporation of these EO into sardine fillets throughout the storage period. Lemon, thyme and oregano essential oils have significant antibacterial activity and may offer a complementary solution for histamine control in fish products.
Key words	CMI, Lemon EO, Thyme EO, Oregano EO, Histamine, Sardine

Effet des huiles essentielles de citron, de thym et d'origan sur la qualité organoleptique et sur la production d'histamine dans les filets de sardine entreposés à 10°C

Résumé	Le recours aux huiles essentielles est considéré l'une des approches émergentes pour préserver la qualité des denrées alimentaires en raison des composés phénoliques à activité antibactérienne qu'elles contiennent. Le pouvoir antibactérien des huiles essentielles de citron, de thym et d'origan a été évalué sur une sélection d'entérobactéries, isolées de sardine avariée, selon la méthode d'aromatogramme. L'influence d'incorporation de ces huiles sur la qualité

organoleptique des filets de sardine, entreposés à 10°C, a été évaluée et le suivi de la teneur en histamine a été réalisé par la méthode fluorométrique de Lerke et Bell. Les huiles essentielles étudiées ont montré une activité antibactérienne importante vis-à-vis de *Klebsiella ozaena*, *Enterobacter cloacae*, *Morganella morganii*, *Proteus mirabilis*, *Citrobacter freundii* et *Providencia rustigianii.* L'huile essentielle (HEs) de citron a montré le diamètre d'inhibition le plus petit tandis que l'HEs de thym a fait apparaitre un diamètre d'inhibition le plus grand. L'application de ces huiles à une concentration de 5% sur les filets de sardines a révélé un effet bénéfique sur les attributs sensoriels et a pu améliorer l'odeur et le goût et préserver la qualité organoleptique de la sardine jusqu'au sixième jour d'entreposage à 10°C sans arriver au seuil de rejet organoleptique. Les huiles essentielles étudiées ont permis le contrôle de la multiplication bactérienne, L'huile essentielle de thym a démontré une diminution marquée de la flore bactérienne d'environ 57%, attestant d'une propriété antibactérienne, tandis que l'utilisation d'huiles essentielles d'origan semblait favoriser un effet bactériostatique. Par ailleurs, l'huile essentielle de citron a contribué à freiner la multiplication des bactéries. Aussi, aucune production d'histamine ne s'est produite suite à l'incorporation de ces HEs dans les filets de sardine durant toute la durée d'entreposage. L'huile essentielle de citron, de thym et d'origan possèdent une activité antibactérienne importante et peuvent constituer une solution complémentaire pour la maitrise d'histamine dans les produits de la pêche.

Mots-clés CMI, HEs Citron, HEs Thym, HEs Origan, Histamine, Sardine

Introduction

Essential oils are natural, liquid, volatile compounds, characterized by a strong odor, extracted from certain plants or plant leaves by steam distillation. They are sparingly soluble or insoluble in water and soluble in typical solvents (ŠKERGET *et al*., 2005). They constitute a mixture of phytochemicals including terpenoids, phenols, and alkaloids that can be used as ingredients to improve shelf life and sensory attributes (BAKKALI *et al*., 2008; EVANS & COWAN, 2016). These phenolic compounds are classified into two categories, phenolic acids derived from cinnamic acid and flavonoids, which are anthocyanins and tannins (DYKES & ROONEY, 2006). The action of these compounds against bacterial growth has been extensively studied, notably for thymol, eugenol and cinnamic aldehyde, which supports their potential use as natural preservatives (LUCERA *et al*., 2012). The FDA has classified terpenes and phenolic compounds as safe fats (BAKKALI *et al*., 2008). The European Commission recognizes limonene, eugenol and p-cymene as safe for human health (DJENNANE, 2015).

Several studies have examined the effect of incorporating essential oils on the quality of seafood products, the effect of rosemary extracts combined with glazing was tested on sardinella stored for 12 days at a temperature of 4°C and concluded a decrease in histamine accumulation (OZYURT *et al*., 2012). The combination of rosemary and sage (*Salvia officinalis*) extracts during storage at 3°C for 20 days of vacuum-packed sardine fillet also had a delaying effect on histamine formation (OZOGUL *et al*., 2011). Applying essential oils of cumin, clove and spearmint to spotted umbrine had the same effect during storage at 4°C for 20 days (CAI *et al*., 2015). NOORI *et al.* (2018) showed that *Carum copticum* extracts could effectively control histamine accumulation during storage of carp fillets stored at 4°C for 18 days. HOUICHER *et al.* (2013) reported that mint essential oils were also effective in controlling histamine accumulation in *Sardina pilchardus* fillets stored at 3°C for 21 days. However, in the majority of studies carried out, the effect of essential oil addition on bacterial behavior was tested either in vitro or on sterile matrices contaminated with a single bacterial strain known in advance and stored at low temperatures (below 4°C), which alone can effectively control bacterial multiplication and histamine accumulation. Also, the results obtained depended to a large extent on the type of plant extract applied, the additional means of preservation put in place, the fish and the storage time, as well as the disadvantages in relation to the strong flavor and limited solubility of essential oils (PRAKASH & KIRAN, 2016). Overall, their efficacy mainly concerned the fate of microbial flora and quality indicators, and no clear trend about the efficacy of histamine control was proposed.

Histamine is a thermostable molecule whose presence in fish indicates that the raw material has been spoiled prior to heat treatment, particularly during the various processing stages which sometimes require relatively long treatment without adequate cold control (filleting, cooling after cooking, maturation of marinades, anchoring, etc.). By evaluating the impact of adding specific essential oils to fresh sardine fillets on histamine production, the evolution of bacterial flora, and the organoleptic and taste characteristics of sardine fillets stored at 10°C—an intermediate temperature where the risk of histamine accumulation is likely—this study aims to support the action of cold applied to fish products during processing.

Materials and Methods

Essential oils used

Ready-to-use essential oils (Concentration 100%/weight) of Oregano, from *Origanum vulgaris*, Lemon, from *Citrus limon*, Clove, from *Eugenia*

caryophyllus, Thyme, from *thymus satureioides, Eucalyptus globulus*, from *Eucalyptus globulus* (Labill), Peppermint, from *Mentha piperita* were ordered from Society "Nouvelle Pharmac", Morocco. To improve their solubility, the oils were used in emulsion form prepared in a mixture of 11% of the oily part and 89% of the aqueous part as described by (MOGHIMI *et al*., 2016). The mixture consists of an addition of (10% w/w) oil, (1% w/w) tween 80 (emulsifier) and (89% w/w) sterile water. The resulting mixture was homogenized by vortexing for 2 min.

In vitro experimental protocol: effect of essential oils on enterobacteria

The efficacy of the essential oils ordered was tested on a selection of bacteria isolated from spoiled sardines, namely *Klebsiella ozaena, Enterobacter cloacae, Morganella morganii, Proteus mirabilis, Citrobacter freundii* and *Providencia rustigiani*, using the aromatogram method, which consists of depositing paper discs previously impregnated with the oil on the surface of a bacterial culture agar, The development of colonies on the surface after incubation reveals blank zones around the disc, known as inhibition diameter. The diameter of this zone depends on the sensitivity of the bacterial strain to the oil tested: the larger the diameter, the more sensitive the bacteria (SOUSSY, 2000). TSA medium was used to refresh the bacterial strains, and Mueller Hinton (MH) medium (Biomérieux® SA-France) was used to test the sensitivity of the bacterial strains to the essential oils studied.

Using the aromatogram method (PONCE *et al*. 2003; SOUSSY, 2000), 4 to 6 identical, well-individualized colonies from each 24h bacterial culture were mixed in 10 ml sterile water, then vortexed for 20 seconds. Next, 1 ml of the resulting suspension was spread onto the surface of MH agar using a sterile loop. Cellulose paper discs were impregnated with the prepared emulsion (10 µl), then placed in the middle of the MH agar inoculated with the bacterial suspension. The plates were then incubated at 35°C for 24 hours. Disks impregnated with sterile water and deposited in MH agar plates inoculated with bacterial suspension were used as a control. The diameter of the inhibition zone was measured in mm using a caliper. Thus, as described by PONCE *et al*. (2003), the bacteria will be qualified as sensitive if the inhibition diameter is between 9 and 14 mm, very sensitive between 15 and 19 mm, extremely sensitive if the diameter exceeds 20 mm and resistant if the diameter is less than 8 mm. The protocol was repeated three times for each EO and each bacterium.

Application on sardine fillets

Two concentrations: 5% and 10% were explored in order to effectively utilize essential oils and manage their potent odour. Sardine fillets containing essential oils of each concentration were subjected to tasting tests by a panel of three specialists. Sardines were to be treated with the 5% concentration. Fresh sardines were gathered at the port of Mehdia, which is situated on the Moroccan Atlantic coast (FAO Zone 34), and sent straight to the food safety laboratory of the Hassan II Agronomic and Veterinary Institute in Rabat, Morocco, to test the antibacterial properties of the essential oils under investigation. The collected sardines were gutted, headed, and separated into four groups (A, B, C, and D) in the lab. Every group was put on a tray and submerged in a 5% emulsion of oregano, thyme, and lemon essential oils. Group D, the control group, received no therapy and was put in a tray. After 30 minutes of immersion, all trays were drained and kept for six days at 10°C in the refrigerator. Three runs of the experiment were conducted and averaged. Every 12 hours for six days of storage following processing and draining, all samples were subjected to the following tests:

Sensory evaluation

The organoleptic characteristics of the sardine fillets were evaluated by a panel of three experienced assessors following the method described by MÖRLEIN (2019), A sliding scale score from 5 to 1 was awarded when assessing rigidity, color, odor, flesh texture and overall appearance, with 5 representing the best score. The average score obtained was then calculated.

Histamine dosage

Histamine content was performed using the fluorometric method of Lerke and Bell (LERKE & BELL, 1976). Histamine extraction was performed on 10 g of well-chopped flesh, to which 90 ml of trichloro-acetic acid (TCA) had been added. Separation was made using an ion exchange column (Amberlite CG50) by chromatography and elution with 0.7 N hydrochloric acid (HCl) (Normality). Histamine levels were read after completion with ortho-phthalaldehyde (OPA) by fluorescence at emission and excitation wavelengths of 450 nm and 360 nm, respectively, using a TrilogyTM fluorometer (Turner Designs Instrument, model 7200-000, California, USA).

Enumeration of bacterial microflora

The evolution of bacterial microflora was examined on samples of sardine flesh taken sterile from each tray. 10g of fish flesh were added to 90 mL of sterile peptone water, ground in a stomacher bag for 1 min. Inoculations were prepared with 0.1mL of stock solution and serial tenth dilutions. Aerobic Plate Count (APC) were counted after double-layer inoculation on Plate Count Agar (PCA) after aerobic incubation at 30°C for 72h, while coliforms and thermo-tolerant coliforms were counted after inoculation on violet red bile lactose (VRBL) after double-layer incubation at 30°C and 44°C respectively for 24h. Enterobacteria were counted on violet red bile glucose (VRBG) medium after incubation in a double layer at 30°C for 24h.

Statistical analysis

The results obtained were studied statistically using the analysis of variance (ANOVA) test with SPSS software, and statistical differences ($p < 0.05$) were determined using R-Studio linear regression software.

Results and Discussion

The efficacy of the essential oils used in this investigation was first assessed on enterobacteria that were isolated from ruined sardines, and then on sardines that had been kept at 10°C for six days. The following are the outcomes of the different tests that were conducted:

In vitro test: Effect of EO on bacteria

The effect of lemon, oregano, thyme, clove, *Eucalyptus globulus* and pepper mint EO was tested on *Morganella morganii, Proteus mirabilis, Klebsiella ozaenae, Providentia rustigianii, Citrobacter freundii* and *Enterobacter cloacae* using the disk diffusion method, determining the inhibition diameter for each bacterial species.

Table 1 reports the results of inhibition diameter measurements for each of the essential oils tested with the bacteria studied.

Table 1: Diameter (in mm) of bacterial inhibition by the essential oils studied
Tableau 1: Diamètre (en mm) d'inhibition des bactéries par les huiles essentielles étudiées

Bacteria	Lemon	Thyme	Oregano	Peppermint	Clove	*Eucalyptus globulus*
Morganella morganii	9	19	15	0	5	0
Proteus mirabilis	13	21	15	0	2	0
Klebsiella ozaenae	10	21	14	0	0	0
Providencia rustigianii	9	20	12	0	0	0
Enterobacter cloacae	8	24	13	0	4	0
Citrobacter freundii	12	19	10	0	2	0

As shown in Table 1, the results of disk diffusion revealed very low zones of inhibition to clove, mint and eucalyptus EO for the bacteria studied, which were resistant to these EO and showed no antibacterial activity compared with lemon, oregano and thyme EO, which showed high antibacterial activity. In fact, all bacteria showed an inhibition diameter indicating a high sensitivity of these bacteria to lemon, thyme and oregano EO. Lemon EO had the smallest inhibition diameter, ranging from 9 to 13 mm. Thyme EO showed the highest inhibition diameter with a value between 19 and 24 mm, while oregano EO showed an inhibition diameter between 10 and 15 mm.

These results are in agreement with several studies which have reported a significant effect antibacterial in vitro of these essential oils and which have demonstrated an inhibitory effect of lemon EO at a dilution of between 0.2% and 0.4% against *staph aureus* and *E.coli* (MOREIRA *et al*., 2005; OUSSALAH *et al*., 2007). BEKHECHI *et al*. (2008) had demonstrated a significant inhibitory effect of oregano EO on *Proteus mirabilis, E. coli, Enterobacter cloacae,* and *Citrobacter freundii*. However, comparing the level of effectiveness of EO to previous studies seems to be difficult, due to the variability of the experimental conditions of each study and the nature of the microorganism used and the dose of EO soaked on the disc. In our study, the essential oils selected for application to sardine fillets were those of lemon, oregano and thyme given their observed antibacterial activity; the other oils were eliminated for their low antibacterial activity.

Effect of EO on sardine quality:

Taste test:

In order to optimize the use of essential oils and to determine the ideal concentration to apply to the sardine matrix which can inhibit bacterial multiplication while controlling the strong characteristic odor of these oils, two concentrations were tested (5 and 10%) by a panel of three experts. Tasting tests of sardine fillets added with essential oils of each concentration, 30 minutes after immersion. The sensory impact of the 5% EO studied was positively reported by the panelists. Indeed, no strong or unpleasant odor was felt for the samples treated with 5% EO compared to those treated with 10% EO, whose strong and characteristic odor of EO was felt on the samples sardine fillets. Thus, the concentration of EO de 5% was chosen for application on sardine fillets.

This study involved monitoring, every 12 hours, the effect of applying three essential oils on sensory attributes, microbiological profile and histamine accumulation in sardine flesh stored at 10°C for six days, compared with a control group not treated with these oils. A total of 144 samples were taken.

Sensory attribute monitoring results:

The sensory impact of the EO studied with a concentration of 5% was positively reported by the panelists. Indeed, and as validated during preliminary tests, no strong or unpleasant odor was felt during the six days of storage for the samples treated with EO at 5% in comparison to those treated with 10% EO whose strong and characteristic odor was felt on the sardine fillets. Thus, the concentration of EO of 5% was chosen for application on sardine fillets. Table 2 shows the evolution of sensory scores for sardines immersed in the essential oils studied, compared with the control, and Figure 1 shows the graph of this evolution. Organoleptic monitoring shows that immersion in lemon, thyme and oregano essential oils preserved acceptable sensory parameters for longer compared with the control group, whose deterioration began around the second day with a loss of brilliance, rigidity and integrity of the flesh, and an unpleasant odor appeared around the third day, culminating in organoleptic

rejection around the fourth day of storage. In samples treated with essential oils, all sensory parameters were well preserved up to the sixth day of storage, without reaching the organoleptic rejection threshold. In addition, a slight odor characteristic of each oil was detected in the samples studied, but this was not a major problem, as the oils used were derived from plants commonly used in Moroccan instead. However, in terms of smell and taste, the panel of tasters unanimously preferred the samples treated with lemon and thyme EO, which they liked to the same degree as those treated with oregano EO. Lemon and thyme EO were able to improve the smell and taste of sardine fillets with the same degree of appreciation. Oregano EO was less appreciated by the panel of tasters.

Table 2: Average sensory scores of sardine fillets treated with lemon, oregano and thyme EO compared with an untreated control group during storage at 10°C for six days.

Tableau 2: Moyenne des scores sensoriels des filets de sardine traités avec les HEs de citron, d'origan et de thym en comparaison avec un groupe témoin non traité durant le stockage à 10°C pendant six jours.

Time sampling (Hours)	0	12	24	36	48	60	72	84	96	108	120	132
Lemon EO	5	5	5	5	4.8	4.4	4.4	3.4	3.4	3.2	3.2	2.6
Thyme EO	5	5	5	5	5	4.2	4	3.4	3.2	3.2	3.2	3
Origano EO	5	5	5	5	4.8	4.6	4.6	4.6	3.8	3.6	3.4	3.2
Control group	5	5	4.6	4	3.2	2.2	1.8	1	0	0	0	0

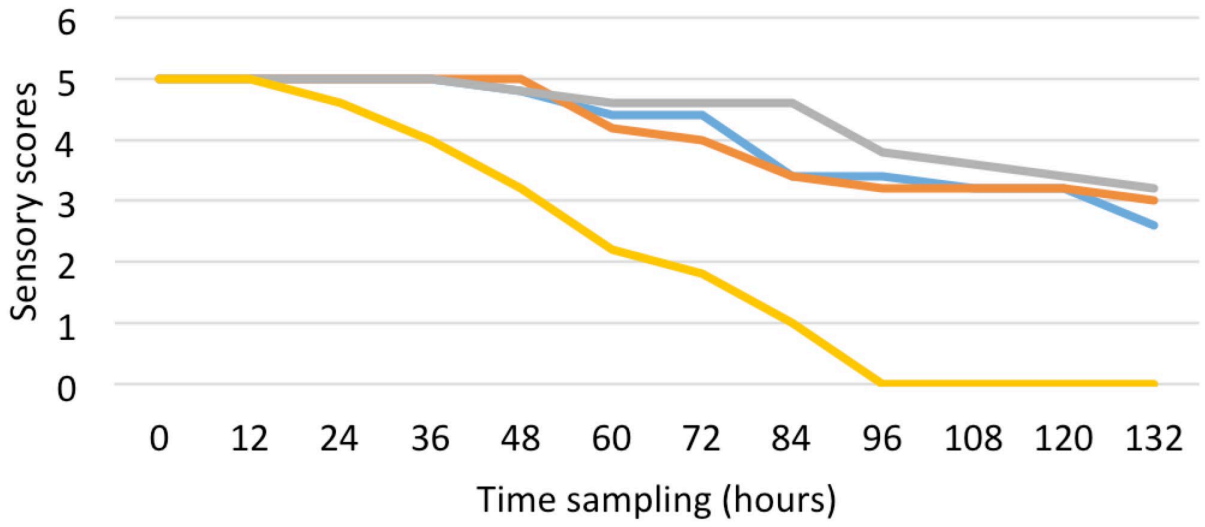

Figure 1: Evolution of mean sensory scores for sardine fillets treated with lemon, oregano and thyme EO compared with an untreated control group during storage at 10°C for six days. Blue: sardine fillets treated with lemon EO, brown: sardine fillets treated with thyme EO, grey: sardine fillets treated with oregano EO, orange: untreated sardine fillets.

Figure 1 : Evolution des moyennes des scores sensoriels des filets de sardine traités avec les HEs de citron, d'origan et de thym en comparaison avec un groupe témoin non traité durant le stockage à 10°C pendant six jour, Bleue : filets de sardine traités à l'HEs de citron, marron : filets de sardine traités à l'HEs de thym, gris : filets de sardine traités à l'HEs de d'origan, orange : filets de sardine non traités.

These results are in line with those reported previously, which have demonstrated a beneficial effect of the use of these essential oils on the organoleptic quality of fish through the continuous release of these oils to the flesh during storage, thus preserving their sensory attributes (SHADMAN *et al.*, 2017; CHUESIANG *et al.*, 2020). In addition, YAZAGAN *et al.* (2017) showed that thyme EO improved the sensory quality of fish fillets GIARRATANA *et al.* (2016) have also reported a positive effect of lemon essential oil on preserving the sensory quality of chilled fish for 15 days.

Bacterial load enumeration results

Table 3 shows the mean of APC count values for samples treated with essential oils and those from the control group.

As shown in Table 3, the low initial bacterial population counts are indicative of a good quality raw material. Monitoring of the APC counts in thyme EO-treated samples revealed a significant reduction in the population over time, from an initial $1.3x10^3$ cfu/g to $0.75x10^3$ cfu/g after six days of storage, with a 57% drop, probably testifying to the bactericidal activity of thyme EO.

A stabilization of the APC population in samples treated with oregano EO was observed, with count values stabilizing at around $1.1x10^3$ cfu/g between the first and

sixth day of storage, in favor of a bacteriostatic action of oregano EO. For samples treated with lemon EO, a moderate increase in the FMAT population was noted, rising initially from $1.2x10^3$ cfu/g to $4.5x10^3$ cfu/g after six days' storage, with a proliferation rate of 3. 7 times, compared with the control group whose APC population proliferated continuously throughout the storage period, multiplying exponentially from an initial $1.2x10^3$ cfu/g to $26.3x10^3$ cfu/g after six days' storage at a 22-fold proliferation rate. Table 4 shows the results of monitoring of the coliform evolution in samples treated with lemon, oregano and thyme EO, compared with the control group.

Table 3: APC load in sardine fillets treated with lemon, oregano and thyme EO compared with an untreated control group during storage at 10°C for six days

Tableau 3: Evolution des FMAT dans les filets de sardine traités avec les HEs de citron, d'origan et de thym en comparaison avec un groupe témoin non traité durant le stockage à 10°C pendant six jours.

Sampling time (Hours)	Lemon EO ($x10^3$ cfu/g)	Thyme EO ($x10^3$ cfu/g)	Oregano EO ($x10^3$ cfu/g)	Control ($x10^3$ cfu/g)
0	1.2	1.3	1.1	1.2
12	1.2	1	1.2	1.9
24	1.2	0.9	1.1	3.6
36	1.5	0.75	0.85	4.4
48	1.7	0.7	0.9	5.9
60	1.6	0.85	1	7.5
72	1.7	0.85	1.1	8.4
84	1.7	1	1.5	8.8
96	3.1	0.85	0.95	12.6
108	4.7	0.85	0.85	16.8
120	4.6	0.7	0.95	21.2
132	4.5	0.75	1.1	26.3

Table 4: Coliform levels in sardine fillets treated with lemon, oregano and thyme EO compared with an untreated control group during storage at 10°C for six days.

Tableau 4: Évolution des coliformes totaux dans les filets de sardine traités avec les HEs de citron, d'origan et de thym en comparaison avec un groupe témoin non traité durant le stockage à 10°C pendant six jours.

Sampling time (Hours)	Lemon EO ($x10^2$ cfu/g)	Thyme EO ($x10^2$ cfu/g)	Oregano EO ($x10^2$ cfu/g)	Control ($x10^2$ cfu/g)
0	0	0	0	0
12	0	0	0	0
24	0	0	0	0.5
36	0	0	0	0.8
48	0	0	0	1.6
60	0	0	0	2.2
72	0	0	0	2.8
84	0	0	0	2.8
96	0	0	0	4.9
108	0.4	0	0	7.5
120	1.1	0	0	10.4
132	2.1	0	0	14.1

Indeed, during the six days of storage, a total absence of coliforms was observed for samples treated with thyme and oregano EO and during the first five days of storage for samples treated with lemon EO, the latter recorded a count value of $0.61x10^2$ cfu/g at the start of the fifth day to multiply 3-fold on the sixth day with a value of $1.8x10^2$ cfu/g. In contrast, the coliform population in the control group multiplied rapidly over the course of storage, rising initially from 0 cfu/g to $15.6x10^2$ cfu/g on the sixth day of storage, with a 156-fold multiplication rate.

Table 5 shows the results of monitoring the evolution of enterobacteria in samples treated with lemon, thyme and oregano EO, compared with the control group.

Table 5: Evolution of Enterobacteriaceae in sardine fillets treated with lemon, oregano and thyme EO compared with an untreated control group during storage at 10°C for six days.

Tableau 5: Évolution des Entérobactéries dans les filets de sardine traités avec les HEs de citron, d'origan et de thym en comparaison avec un groupe témoin non traité durant le stockage à 10°C pendant six jours.

Sampling time (Hours)	Lemon EO ($x10^2$ cfu/g)	Thyme EO ($x10^2$ cfu/g)	Oregano EO ($x10^2$ cfu/g)	Control group ($x10^2$ cfu/g)
0	0	0	0	0
12	0	0	0	0
24	0	0	0	0.5
36	0	0	0	0.8
48	0	0	0	1.6
60	0	0	0	2.2
72	0	0	0	2.8
84	0	0	0	2.8
96	0	0	0	4.9
108	0.4	0	0	7.5
120	1.1	0	0	10.4
132	2.1	0	0	14.1

Enterobacteriaceae were absent throughout storage for thyme and oregano EO-treated samples, and during the first four days of storage for lemon EO-treated samples, which recorded a count value of $0.4x10^2$ cfu/g by the end of the fifth day, multiplying 5.2-fold on the sixth day with a value of $2.1x10^2$ cfu/g. In contrast, the enterobacteria population in the control group multiplied rapidly throughout storage, rising from 0 cfu/g initially to $14.1x10^2$ cfu/g on the sixth day of storage, with a 141-fold multiplication rate.

The essential oils used in this study allowed the control and stabilization of bacterial multiplication in sardine fillets during six days of storage following the inhibition of the bacteria through the physicochemical modification of the medium. Previous studies have reported higher microbial counts of approximately $6x10^6$ cfu/g during storage at 4°C (JINADASA *et al*., 2015). In the three immersion tests for lemon, oregano and thyme EO, the admissible threshold was not reached and the values found after the sixth day of storage were well below the regulatory threshold. The microflora of samples treated with thyme and oregano EO was lower than that of samples treated with lemon EO. These results are consistent with those reported previously which demonstrated antimicrobial activity of EO against bacteria. Indeed, a significant reduction in the bacterial population was reported when applying oregano and thyme EO in food matrices. (GHADERI-GHAHFAROKHI *et al*., 2016; MORAES-LOVISON *et al*., 2017). Other authors had reported a bactericidal effect of thyme EO inducing a reduction Aerobic Plate Count. (SHERIFF MAQBUL *et al*., 2020; TORRES NETO *et al*., 2022; MILAGRES DE ALMEIDA *et al*., 2023). Also, ALFONZO *et al*. (2017) had reported a preservative effect of lemon essential oil with an inhibitory action on enterobacteria, KUNOV *et al*. (2021) had observed an inhibitory effect on coliforms on samples treated with these oils. The mechanism of action of these EO has been addressed in several studies, in fact, EO can interfere with the protein components of the cell membrane of bacteria, thus preventing their proper development.

Effect of EO on histamine content

Table 6 represents the evolution of histamine accumulation in sardine samples immersed in lemon, thyme and oregano EO, and Figure 2 schematizes this evolution.

The essential oils studied made it possible to effectively control the induction of histamine in the sardine, no histamine production occurred in the samples immersed in the EO. Indeed, the values recorded in histamine were significantly lower compared to those of the control group.

The initial level of histamine in the sardine flesh was negligible. Immersion of the sardine in the three essential oils caused a total inhibition of histamine production during the six days of storage at 10°C. The average histamine levels were between 0.45 and 6.1 ppm for the samples treated with lemon EO, and between 0 and 32.45 ppm for those treated with thyme EO and between 1.05 and 5.41 ppm for those treated with Oregano EO. The values of the control group were significantly higher and ranged from 1.03 to 208.23 ppm. The regulatory threshold for histamine was not reached for the samples treated with EO throughout the storage period with a very high level of safety, while those in the control group exceeded the regulatory threshold on the fourth day of storage. The results obtained were in agreement with those reported by ALFONZO *et al*. (2017).

Table 6: Evolution of the histamine level in sardine fillets treated with lemon, oregano and thyme EO in comparison with an untreated control group during storage at 10°C for six days.

Tableau 6: Evolution du taux d'histamine dans les filets de sardine traités avec les HEs de citron, d'origan et de thym en comparaison avec un groupe témoin non traité durant le stockage à 10°C pendant six jours.

Sampling time (Hours)	Lemon EO (ppm)	Thyme EO (ppm)	Oregano EO (ppm)	Control group (ppm)
0	5.03	1.25	3.14	1.03
12	3.36	4.83	4.56	3.12
24	2.16	2.56	2.78	7.45
36	3.21	4.16	3.59	13.56
48	1.91	1.35	1.81	14.56
60	4.12	2.86	3.76	25.4
72	2.85	0	3.75	35.4
84	5.4	0	3.87	60.5
96	6.1	4.6	5.41	99.12
108	4.52	3.25	1.05	133.48
120	2.45	2.89	3.65	170.25
132	0.45	32.45	2.05	208.23

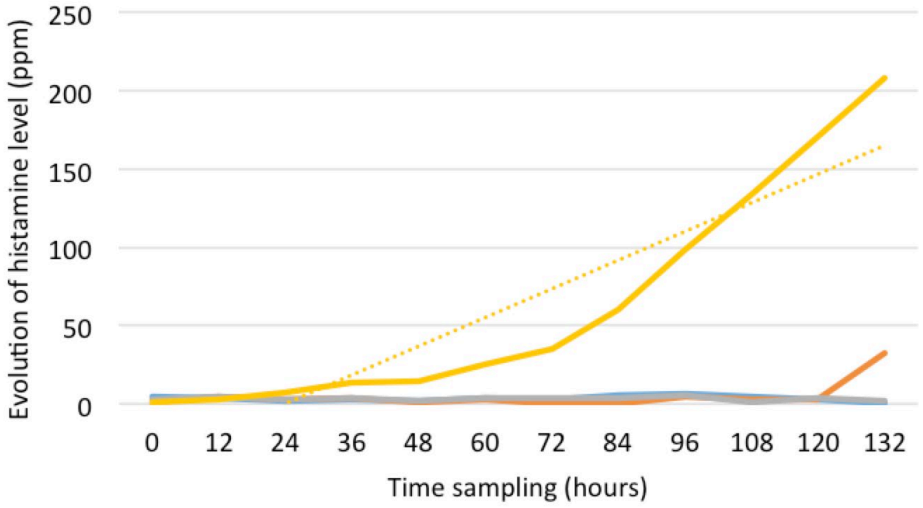

Figure 2: Evolution of the histamine level in sardine fillets treated with lemon, oregano and thyme EO in comparison with an untreated control group during storage at 10°C for six days. Blue: sardine fillets treated with lemon EO, brown: sardine fillets treated with thyme EO, gray: sardine fillets treated with oregano EO, orange: untreated sardine fillets.

Figure 2 : Evolution du taux d'histamine en ppm dans les filets de sardine traités avec les HEs de citron, d'origan et de thym en comparaison avec un groupe témoin non traité durant le stockage à 10°C pendant six jour, Bleu : filets de sardine traités à l'HEs de citron, marron : filets de sardine traités à l'HEs de thym, gris : filets de sardine traités à l'HEs de d'origan, orange : filets de sardine non traités.

As shown in Figure 3, the analysis of variance of the effect of adding the three EO on the sardine fillets, carried out with the ANOVA test, revealed a significant effect ($p < 0.001$) of the incorporation of these additives compared to the control group during the storage period in relation to the dependent variables relating to microbial counts, histamine levels and sensory scores, the comparison of the means between the groups treated with EO between them revealed no difference significant.

Linear regression analysis for APC revealed an R square value of 0.4813, meaning that 48.13% of the variability in APC counts could be explained by the incorporation of EO. Indeed, treatment with EO of Lemon, Oregano and Thyme reduced the APC respectively by 7.5 $x10^3$ CFU/g, 8.8 $x10^3$ cfu/g and 9 $x10^3$ cfu/g units compared to the control (without treatment).

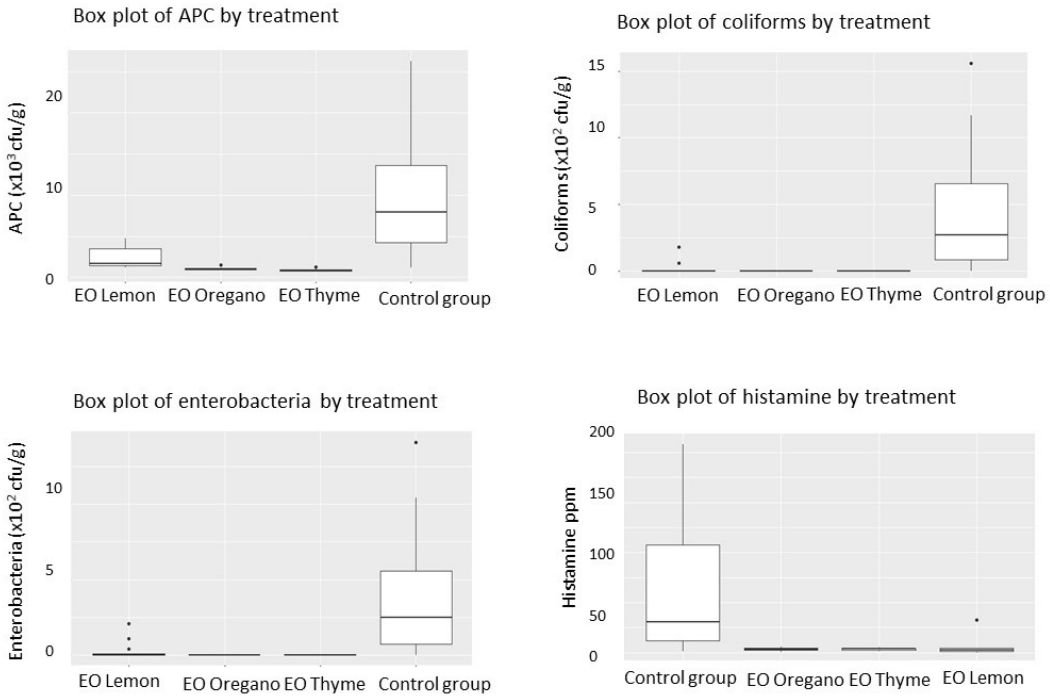

Figure 3: Analysis of variance of the evolution of APC, Coliform, Enterobacteria and Histamine

Figure 3 : Analyse de variance de l'évolution des FMAT, CT, Entérobactéries et de l'Histamine.

Conclusion

The work addressed in this study highlighted the antibacterial activity of lemon, thyme and oregano EO. Indeed, these essential oils had significant antibacterial activity against bacteria isolated from sardines. The satisfactory results of the evolution of the sensory parameters of the samples treated with these essential oils led to the preservation of the organoleptic quality of the sardine stored at 10°C compared to the untreated one. Also, the remarkable antibacterial effect which was revealed at the level of counting the population of APC, Coliform and Enterobacteria, allows us to conclude that the use of these essential oils can play a primordial antibacterial role and can constitute a solution for the histamine control in fish products. The economic impact of our results will be very beneficial to the extent that the treatment with EO will have a positive effect on improving the sensory quality of the sardine, in particular, the distinctive flavor provided by these EO which could significantly encourage the consumption of sardines. .

Other perspectives can be explored through more in-depth studies of the effect of mixing EO in search of possible synergy and complementarity between their effects, to extend the study to other bacterial strains recognized as highly histaminogenic to confirm or refute the effectiveness of EO in controlling histamine production. Also, the incorporation of EO into bioactive packaging films to control the accumulation of histamine in cans of canned and semi-canned fish rich in histidine would also be an avenue to explore.

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