INVESTIGACIÓN

Antifungal activity of essential oils when associated with sodium chloride or fatty acids

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RESUMEN

Actividad antifúngica de aceites esenciales en asociación con cloruro sódico o ácidos grasos.

Se ha estudiado la inhibición del crecimiento miceliar en un aislamiento de *Zygorhynchus sp.* y otro de *Aspergillus niger.* Se determinaron las tasas (o índices) de inhibición (IR) en Agar Sabouraud Dextrosa provocados por varias concentraciones de 4 aceites esenciales (EO), 5 ácidos grasos y cloruro sódico.

Se observó un efecto sinérgico entre cloruro sódico al 7.5% y los aceites esenciales de tomillo (0.04%), manzanilla (0.4%) y artemisa (0.2 y 0.1%) sobre *A. niger,* y entre cloruro sódico (5%) y los aceites esenciales de manzanilla (0.1%) y de artemisa (0.1 y 0.01%), así como cloruro sódico (7.5%) y aceite esencial de eucalipto (0.4 y 0.2%), sobre *Zygorhynchus sp.*

El aceite esencial de manzanilla (0.13%) en asociación con ácido propiónico (0.075%), ácido láurico (0.05%) o ácido oleico (0.15%) provocó un efecto sinérgico sobre *Zygorhynchus sp.*, de la misma forma que aceite esencial de tomillo (0.04 y 0.05%, respectivamente) con ácido propiónico (0.1%) y ácido linolénico (0.075%) sobre *A. niger.* Otras combinaciones no produjeron efectos mayores que los de cada sustancia aisladamente.

Se discuten las aplicaciones prácticas de los resultados observados.

PALABRAS-CLAVE: Aceite esencial — Ácido graso — Actividad antifúngica — Cloruro sódico.

SUMMARY

Antifungal activity of essential oils when associated with sodium chloride or fatty acids.

The inhibition of mycelium growth in a *Zygorhynchus sp.* and an *Aspergillus niger* isolates was studied. The inhibition rates (IR) caused by 4 essential oils (EO), 5 fatty acids and sodium chloride at various concentrations were determined in Sabouraud Dextrose Agar.

A synergy of action was observed between sodium chloride at 7.5% and the EO of thyme (0.04%), camomile (0.4%) and mugwort (0.2 and 0.1%) on *A. niger* and between sodium chloride (5%) and the EO of camomile (0.1%) and mugwort (0.1 and 0.01%) and sodium chloride (7.5%) and eucalyptus EO (0.4 and 0.2%) on *Zygorhynchus sp.*

Camomile EO (0.13%) associated with propionic acid (0.075%), lauric acid (0.05%) or oleic acid (0.15%) led to synergetic effect on *Zygorhynchus sp.* as well as thyme EO (0.04 and 0.05%, respectively) with propionic acid (0.1%) and linolenic acid (0.075%) on *A. niger.* Other combinations exerted no higher effects than each of the substances used alone.

Practical applications of the results observed were discussed.

KEY-WORDS: Antifungal activity — Essential oil — Fatty acid — Sodium chloride.

1. INTRODUCTION

The antifungal activity of plant essential oils (EO) is well documented (Dayal and Purohit, 1970; Narasimha and Subbarao 1972; Malles *et al.*, 1979; Benjilali *et al.*, 1984). It even has been reported that EO have inhibitory effects against microbial development in food products (Dabbah *et al.*, 1970; Bullerman *et al.*, 1977). Thus, food preservation with EO has been envisaged (Jurd *et al.*, 1971; Busta and Foegeding, 1983; Conner and Beuchat, 1984).

Moulds are also sensitive to fatty acids (FA) (Hellgren and Vincent, 1972; Jay, 1986). Some of them such as propionates are used in food preservation and it is established that their activity is mainly directed towards moulds (Sauer, 1977; Rusul *et al.*, 1987; Beuchat and Golden, 1989; Wagner and Moberg, 1989).

Sodium chloride is used in a wide variety of foods either to enhance their taste or to protect them against microorganisms. However, its antifungal potentialities are weak as compared to its activity on bacteria (Jay, 1986).

Whether intentionnally added or introduced with spices and herbs, EO may co-exist in some foods together with sodium chloride or fatty acids. The aim of this study was to determine the antifungal activity of selected EO when combined with sodium chloride and fatty acids.

The EO from thyme *Thymus broussonettii,* wild camomile *Ormenis mixta L.*, mugwort *Artemisia herba alba*, and eucalyptus *Eucalyptus globulus* were used in this study. They were chosen because of the different nature of their major components; respectively a phenol (carvacrol: 47.8% of total oil), alcohols (Santolina alcohol: 37%, artemisia alcohol: 2,2%), ketones (camphor: 48.1%, α-thujone: 4.1%, β-thujone: 1.8%) and an oxide (1,8 cineole: 76.4%).

Since the antimicrobial activity of FA is related to their chain length and unsaturation level (Nieman, 1954; Foster and Wynne, 1984), six FA were selected according to those two parameters. These were propionic (C3:0), lauric (C12:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids.

2. MATERIALS AND METHODS

2.1. The antifungal agents used

Pure analytical quality sodium chloride was used.

EO extraction and analysis procedures have been described previously (Tantaoui-Elaraki *et al.*, 1992).

All FA were purchased from NU-CHEK-PREP, Inc. (USA). They were provided in sealed vials with 99%

minimun purity. Unsaturated FA were conditioned under vacuum. The FA were saponified with a Millipore-sterilized sodium hydroxide solution and used as sodium salts at pH 7.3.

2.2. The microorganisms

Two mould isolates were used. One belonged to the species *Aspergillus niger*, an imperfect form of an Ascomycete and a common contaminat of low water content foods. It had been isolated in our laboratory from "Greek style" black olives. The other belonged to the genus *Zygorhynchus*, a Zygomycete that grows well on relatively high water content foods. This isolate has been kindly provided by the Mycology Lab., Indiana University (USA).

The inocula were prepared from 7 day cultures at 25°C on Sabouraud Dextrose Agar (SDA). The spores were suspended in a 8.5g/l NaCl solution containing 0.1% tween 80 to stimulate spore dispersion. Their number was adjusted by dilution to 10^6 spores/ml.

2.3. Preparation of the growth medium with the antifungal agents

Sodium chloride was added to SDA at 5%, 7.5%, 10%, 12.5%, 15% and 20%.

The EO were dispersed in 0.1% Tween 80 water solution and incorporated into the medium as described previously (Tantaoui-Elaraki *et al.*, 1992). When alone, the EO were tested at 0.01%, 0.1%, 0.2%, 0.4% and 1%. Other proportions were used if necessary when the EO were to be associated with other substances.

The FA were added at different concentrations to test tubes containing 15ml of 4/3 reinforced SDA; i.e. SDA with 4/3 the concentrations of its components. The tubes were then completed to 20ml with sterile distilled water so as the final amounts of the nutrients were those of normal SDA with the following FA concentrations: 0.025%, 0.05%, 0.075%, 0.1%, 0.15%, 0.2%, 0.25% and 0.3%. Other concentrations were also used in some combinations with EO.

2.4. Experimental procedure

The aim of the experiment was to study the antifungal effect of the EO when combined with sodium chloride, or fatty acids. Consequently it was first necessary to determine the concentration of each substance that will lead to a partial inhibition of each fungus. Then an EO and another substance among the ones investigated were combined at their respective partially inhibitory concentrations in order to make evidence of any synergy or antagonism of action.

2.5. Determination of the inhibition rates caused by individual antifungals

The media prepared with different concentrations of antifungal agents as indicated above were poured into Petri dishes (20ml per dish) and surface-inoculated with 0.1ml of spore suspension. Then they were incubated at 25°C for 7 d, together with the control (SDA). At the end of the incubation period, the spores were killed by a short heat treatment (120°C, 30 sec.) and the mycelia were recovered, washed with distilled water to remove the medium components and dried at 90°C for 18hr. The trials were repeated 3 times and the average mycelium dry weights calculated. The inhibition rate (IR) was then determined as follows:

$$IR = \frac{DWc - DWt}{DWc} \times 100$$

IR: Inhibition rates (% of inhibition)
DWc: Mycelium dry weight in the control
DWt: Mycelium dry weight in the test

2.6. Choice of the combinations

Each combination will be defined by the fungus involved, the nature and concentration of the EO and the nature and concentration of the substance (sodium chloride or FA) to be associated with the EO. The choice of the combinations to be investigated was based upon the IR observed with each antifungal agent tried individually.

2.7. Antifungal effects of the combinations

For each combination, 4 Petri dishes were prepared one with SDA containing the EO at the suitable concentration, one with SDA containing the other substance at the concentration chosen, one with both (EO + the other substance) and one control with normal SDA. However, all Petri dishes had the same amount of Tween 80 whether they contained EO or not, and they all were adjusted to pH 7.3 regardless of the presence of FA.

The Petri dishes were inoculated and incubated as indicated above and the IR was determined. All trials were done in triplicate, and the average IR of the 3 determinations were taken into account.

Statistical tests were run including variance analysis and the calculation of the Lowest Significant Difference (LSD) in order to determine whether the action of the two substances used together was significantly different from the effect of each one tried separatelly, and also to compare fungal growth in presence of the antifungal agents to the control.

3. RESULTS AND DISCUSSION

3.1. The inhibition rates caused by individual substances

Tables I and II contain the IR caused by the EO on *A. niger* and *Zygorhynchus sp.*, respectively. Thyme essential oil (TEO) turned out to be the most efficient, and eucalyptus (EEO) the least active as already shown by another technique (Tantaoui-Elaraki *et al.*, 1992). Camomile oil (CEO) had more efficacy than mugwort essential oil (MEO) at 0.4% on *Zygorhynchus sp.* (Table II). Otherwise, it was slightly less active on both fungi. The relative efficacy of the four EO studied ranks with the rule established by some authors such as Kurita *et al.* (1981) according to which phenolic oils should be more active on microorganisms than alcoholic, ketonic and oxidic ones.

Only the relative ranking of CEO and MEO does not match exactely this rule, probably because of the influence of minor components of the oils as already suggested (Tantaoui-Elaraki *et al.*, 1992).

Table I. **Inhibition rates observed on an *Aspergillus niger* isolate by 4 essential oils at different concentrations in Sabouraud Dextrose Agar**

Concentrations	TEO	CEO	MEO	EEO
1 %	100	100	100	51.9
0.4 %	100	26	68	15.6
0.2 %	100	8	26.5	-2.3
0.1 %	100	-6	6.5	-11
0.01 %	-0.3	-23	-8.7	-43.5

TEO: Thyme essential oil
CEO: Camomile essential oil
MEO: Mugwort essential oil
EEO: Eucalyptus essential oil

Table II. **Inhibition rates observed on a *Zygorhynchus sp.* isolate by 4 essential oils at different concentrations in Sabouraud Dextrose Agar**

Concentrations	TEO	CEO	MEO	EEO
1 %	100	100	100	92.8
0.4 %	100	100	68	58.8
0.2 %	100	56.6	72.8	22.9
0.1 %	100	14.5	23.2	2.5
0.01 %	-1.5	6.7	11.4	-32

TEO: Thyme essential oil
CEO: Camomile essential oil
MEO: Mugwort essential oil
EEO: Eucalyptus essential oil

Zygorhynchus sp. showed more sensitivity than *A. niger,* which corroborates previous results with these two strains exposed to various essences (Tantaoui-Elaraki *et al.*, 1992; Hmamouchi *et al.*, 1990).

A growth stimulation phenomenon was observed in presence of low amounts of EO. With EEO, this was noticed on *A. niger* with concentrations as high as 0.2%, but the lower the concentration, the stronger was this stimulation (Table I).

Tables III and IV include the IR caused by the FA on *A. niger* and *Zygorhynchus sp.*, respectively. It was lauric acid that exerced the highest inhibitory effect on both fungi, followed by propionic acid. Within the C18 fatty acids, the higher the unsaturation level, the more active was the acid, which confirms previous observations (Kodicek and Worden, 1946; Galbraith *et al.*, 1971). The FA were used in this study as sodium salts at pH 7.3, which probably reduced their efficacy since antifungal acids are more active at low pH (Jay, 1986). This is because apart from propionic acid, the FA tested are not soluble in water, and we failed to emulsify them in 0.1% and 0.2% Tween 80 solutions. Also sodium desoxycholate at 0.1% was able to disperse them but this substance was inhibitory to Gram positive bacteria that were used in a parallel study.

Table III. **Inhibition rates observed on an *Aspergillus niger* isolate by six fatty acids at different concentrations in Sabouraud Dextrose Agar**

Concentrations (g/100 ml)	C3:0	C12:0	C18:0	C18:1	C18:2	C18:3
0.025	1.8	20.3	-14.3	-1	5.4	13
0.050	7.4	55.5	-9.3	6	19.4	12.9
0.075	11.9	98.7	-	-	-	-
0.100	34.8	100	7.2	10.6	36.3	54
0.150	94.6	100	15.3	18.4	51.9	85.2
0.200	100	100	28	11.6	78	97.3
0.250	100	100	45	50.2	74.9	100
0.300	100	100	-	48	97.3	100

C3:0 = Propionic acid
C12:0 = Lauric acid
C18:0 = Stearic acid
C18:1 = Oleic acid
C18:2 = Linoleic acid
C18:3 = Linolenic acid

Table IV. **Inhibition rates observed on a *Zygorhynchus sp.* isolate by six fatty acids at different concentrations in Sabouraud Dextrose Agar**

Concentrations (g/100 ml)	C3:0	C12:0	C18:0	C18:1	C18:2	C18:3
0.025	3.1	13.4	-16.1	2.4	-	3.1
0.050	8.0	34.9	-9.3	7.1	-	8.5
0.075	33.5	75.7	0	-	-	-
0.100	84.9	99.9	1.8	14.3	-	40.6
0.150	100	100	1.8	52.0	-	48.6
0.200	100	100	11.5	60.0	38.5	71.9
0.250	100	100	13.2	61.0	54	100
0.300	100	100	20.5	62.6	69.7	100

C3:0 = Propionic acid
C12:0 = Lauric acid
C18:0 = Stearic acid
C18:1 = Oleic acid
C18:2 = Linoleic acid
C18:3 = Linolenic acid

Some FA, mainly stearic acid, induced a growth stimulation at low concentrations in both fungi. This corroborates previous results reported by various authors (Nieman, 1954; Chander *et al.*, 1979).

The IR of NaCl on *A. niger* was 16.5% with a concentration of 7.5%. With higher NaCl concentrations, the IR became much higher (Table V). Sodium chloride at 5% stimulated *A. niger* growth, which is in accordance

with the observations of Rai and Agarwal (1974) that optimum NaCl concentrations are 3% and 5% for halotolerant and holophilic Aspergilli, respectively.

No stimulation effect was noticed on *Zygorhynchus sp.* A slight inhibition was observed with 5% NaCl and the IR was 100% starting from 12.5% NaCl (Table V).

Table V. **Inhibition rates observed on a *Zygorhynchus sp.* and an *Aspergillus niger* isolates by sodium chloride at different concentrations in Sabouraud Dextrose Agar**

	Sodium chloride concentrations (%)					
	5	7.5	10	12.5	15	20
Zygorhynchus sp.	7	65.6	83.8	100	100	100
A. niger	-27.1	16.5	62.8	73.8	97.8	100

3.2. Choice of the combinations to be investigated

The objective in this section was to look for synergistic actions. Thus, the concentrations to be used in a combination were preferably those separately leading to an IR lower than 40% so as an IR of 100% or so in the combination would mean a synergy of action. For example for *A. niger*, the salt concentration adopted was 7.5% and for *Zygorhynchus sp.* we used either 5 or 7.5% (see Table V). Sometimes, the concentration to be used in a combination was extrapolated from the results recorded. For example, TEO was used at 0.04 and 0.05% which should give suitable IR according to the results noticed for 0.1 and 0.01% (see Table I); i.e. 100% and 0.3%, respectively.

3.3. Effects of the combinations of sodium chloride with essential oils

The effects of the combinations of NaCl at 7.5% with different EO on the growth of *A. niger* are presented in table VI.

When TEO was used at 0.04% the LSD was 43.4, which means that NaCl alone exerted no significant effect while TEO did. However, the effect, was greatly enhanced when sodium chloride and TEO were associated.

With CEO, the LSD was 57.4; while neither NaCl nor CEO had a significant effect, the combination of both led to complete growth inhibition.

The individual effect of MEO on *A. niger* depended upon the concentration used. At 0.2%, MEO had no significant inhibitory action, the LSD being 60.5, while at 0.1%, this oil led to a mycelium dry weight non significantly different from the control, the LSD being 63.3. The combination of MEO with NaCl at 7.5% led to total inhibition of fungal growth regardless of the concentration of the oil.

The effects of the combinations of NaCl with EO on the growth of *Zygorhynchus sp.* are summarized in table VII.

The effect of sodium chloride alone either at 5% or at 7.5% was not significant in any case.

CEO at 0.1%, MEO at 0.1% and EEO at 0.4% exerted significant antifungal effects, the LSD being 34.8, 30.6 and 31.1, respectively. MEO at 0.01% and EEO at 0.2% however showed no significant effects, the LSD being 33.1 and 30.1, respectively.

In all cases, the combinations of NaCl with the EO led to significant inhibitory effects and when the EO alone was active, NaCl addition increased significantly its effect.

On both fungal strains used, it is clear that a synergy of action exists between sodium chloride and the EO investigated. The IR calculated for the combinations were always higher than the addition of those obtained separately for NaCl and the EO alone.

A similar synergy of action has been observed between oregano EO at 1% and NaCl at 3% on several moulds (Akgül and Kivanç, 1988). Also Kurita and Koike (1982, 1983) have shown a synergetic activity of sodium chloride with some EO components on microorganisms. However, the intensity of such a synergy was different from one component to another; e.g. the effect was much stronger with linalool than 1,8 cineole.

In this study, the EO were used as such and the synergetic effects observed could not be easily explained since any of the EO components whether major or minor could be involved.

Table VI. **Effects of the combinations of NaCl (7.5%) with essential oils on the growth of an *Aspergillus niger* isolate incubated for 7d at 25ºC on Sabouraud Dextrose Agar. The figures represent the mycelium dry weights (in mg) and the inhibition rates (in brackets).**

Essential oil nature and concentration	Control	NaCl alone	E.O alone	NaCl+E.O	LSD
TEO; 0.04 %	248.2*	233.2 (6.0)	193.8 (21.9)	4.5 (98)	43.4
CEO; 0.4 %	187.9	176.6 (6.0)	130.6 (30.4)	0 (100)	57.4
MEO; 0.2 %	187.9	176.6 (6.0)	138.6 (26.2)	0 (100)	60.5
MEO; 0.1 %	187.9	176.6 (6.0)	175.5 (6.5)	0 (100)	63.3

* All figures are averages of 3 determinations
EO: Essential oil
TEO: Thyme essential oil
CEO: Camomile essential oil
MEO: Mugwort essential oil
LSD: Least Significant Difference

Table VII. **Effects of the combinations of NaCl with essential oils on the growth of a *Zygorhynchus sp.* isolate incubated for 7d at 25ºC on Sabouraud Dextrose Agar. The figures represent the mycelium dry weights (in mg) and the inhibition rates (in brackets).**

Nature of the combinations	Control	NaCl alone	E.O alone	NaCl+E.O	LSD
NaCl; 5 % CEO; 0.1 %	186.6*	182.3 (2.1)	147.7 (20.8)	33 (82.3)	34.8
NaCl; 5 % MEO; 0.1 %	186.6	182.3 (2.1)	135.1 (27.6)	11 (94.1)	30.6
NaCl; 5 % MEO; 0.01%	190.5	178.3 (6.4)	181.9 (4.5)	98.8 (48.1)	38.1
NaCl; 7.5 % EEO; 0.4 %	184.7	186.7 (1)	120 (35.0)	0 (100)	31.1
NaCl; 7.5 % EEO; 0.2 %	176.8	166.8 (4.7)	148 (16.3)	27 (48.7)	30.1

* All figures are averages of 3 determinations
EO: Essential oil
MEO: Mugwort essential oil
CEO: Camomile essential oil
EEO: Eucalyptus essential oil
LSD: Least Significant Difference

3.4. Effects of the combinations of fatty acids with essential oils

The effects of the combinations of selected fatty acids with camomile essential oil (CEO) on the growth of *Zygorhynchus sp.* are presented in Table VIII.

Propionic acid at 0.075% and CEO at 0.13% exerted a significant inhibition on the mould (LSD 48.7). However, the effect of the combination was much higher, the IR being 100% instead of 34.6 and 4%, for the FA and the EO, respectively, when used alone.

The combination of lauric acid at 0.05% with CEO at 0.13% led to a synergetic action too. However, the LSD being 82.4 the EO was significantly inhibitory when used alone while the FA had no significant effect.

In the assay with stearic acid at 0.15% and CEO at 0.13% none of the antifungals had any significant effect when they were tried separately. However, their combination turned out to be significantly efficient, the LSD being 101.6.

A different pattern was observed with the combination of linoleic acid at 0.1% with CEO at 0.1%. In this case, neither of the substances tested alone was able to induce any significant inhibition. Moreover, the combination led to a negative IR, which means that it somehow increased the growth of the fungus. However, the LSD was as high as 114.4, which shows that there were no significant differences between the mycelium dry weights determined.

The effects of the combinations of selected fatty acids with thyme essential oil (TEO) on the growth of *A. niger* are summarized in table IX.

In the case of propionic acid at 0.1% with TEO at 0.04% and also in the trial with linolenic acid at 0.075% with TEO at 0.05%, only the combinations FA+EO led to significant inhibitory effects on the growth of the fungus, the LSD being 100.2 and 133.7, respectively.

The combination of lauric acid at 0.03% with TEO at 0.04% seemed to increase fungal growth (IR:-4.2). However, there was no significant differences between the mycelium dry weights, the LSD being 38.0.

A synergetic antifungal activity could be observed in this study with several combinations between FA and EO (Tables VIII and IX). Few data are available in the literature regarding antimicrobial effects of EO in combination with FA. A synergy of action has been reported between acetic acid and EO components (Kurita and Koike, 1983). Also Lattaoui (1985) has mentionned a presumptive synergy of propionic acid with TEO and EEO on the bacterium *Staphylococcus aureus* and the fungus *Aspergillus flavus.*

However, the mechanism of such a synergy is still unknown. Many investigations have led to the conclusion that FA cause mainly disturbances in the microbial cell membrane (Sheu and Freese, 1972; Galbraith and Miller, 1973; Greenway and Dyke, 1979; Beuchat and Golden, 1989). The EO have been shown to cause multisectorial cell disorders in microorganisms (Franchomme, 1981), with disturbances in the ionic state of cell membrane in fungi (Kurita *et al.*, 1979). Also some EO components have been shown to deeply damage fungal cell membrane (Bard *et al.*, 1988).

The synergy of action between EO and sodium chloride and/or FA may take place in some foods like Moroccan smen which is a lipolysed fat made from butter that is

Table VIII. **Effects of the combinations of selected fatty acids with camomile essential oil on the growth of a *Zygorhynchus sp.* isolate incubated for 7d at 25ºC on Sabouraud Dextrose Agar. The figures represent the mycelium dry weights (in mg) and the inhibition rates (in brackets).**

Nature of the combinations	Control	F.A alone	E.O alone	F.A+E.O	LSD
C3:0; 0.075 % CEO; 0.13 %	268.4*	175.5 (34.6)	158.2 (41)	0 (100)	48.7
C12:0; 0.05 % CEO; 0.13 %	279.5	227.8 (18.5)	184.8 (33.8)	22.1 (92)	82.4
C18:0; 0.150 % CEO; 0.13 %	251.4	249.1 (0.9)	170.3 (32)	43.2 (82.8)	101.6
C18:2; 0.100 % CEO; 0.1 %	288.2	270.6 (6.1)	216.6 (24.8)	295.1 (-2.3)	114.4

* All figures are averages of 3 determinations
C3:0 = Propionic acid
C12:0 = Lauric acid
C18:0 = Stearic acid
C18:2 = Linoleic acid
FA: Fatty acid
EO: Essential oil
CEO: Camomile essential oil
LSD: Least Significant Differences

Table IX. **Effects of the combinations of selected fatty acids with Thyme essential oil on the growth of an *Aspergillus niger* isolate incubated for 7d at 25ºC on Sabouraud Dextrose Agar. The figures represent the mycelium dry weights (in mg) and the inhibition rates (in brackets).**

Nature of the combinations	Control	F.A alone	E.O alone	F.A+E.O	LSD
C3:0; 0.100 % TEO; 0.04 %	245.8*	199.1 (19)	205.9 (16.2)	-	100.2
C18:3; 0.075 % TEO; 0.05 %	390.6	344.9 (11.7)	312 (20.1)	64.3 (83.5)	133.7
C12:0; 0.030 % TEO; 0.04 %	326.6	301.4 (7.7)	289.6 (11.3)	340.6 (-4.2)	38.0

* All figures are averages of 3 determinations
C3:0 = Propionic acid
C12:0 = Lauric acid
C18:3 = Linoleic acid
FA: Fatty acid
EO: Essential oil
TEO: Thyme essential oil
LSD: Least Significant Differences

washed, salted and stored at room temperature for several months during which time lipolytic microorganisms release free FA that develop the specific taste and aroma of the product (Tantaoui-Elaraki and El Marrakchi, 1987). In some areas, it is common practice to use aromatic plants such as thyme or mugwort in order to enhance smen aroma.

This synergy could also be used in other foods where the addition of selected EO together with sodium chloride and/or chosen FA should not alterate the organoleptic characteristics since the concentrations needed would be very low.

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