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Antimicrobial effect of encapsulated and non-encapsulated thyme essential oil

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Some plants contain functional compounds with antimicrobial activity. Phenols, polyphenols, micronutrients and essential oils belong to them. These bioactive compounds can be used against pathogenic and food spoilage bacteria. Thyme essential oil belongs to the plant material with a powerful antimicrobial activity. The aim of the study was to test the antimicrobial effect of thyme essential oil and polyethylene foil, which is coated with partially water soluble polymeric film containing encapsulated thyme essential oil on selected microorganisms with or without direct contact. *Escherichia coli*, *Candida tropicalis* and *Penicillium chrysogenum* were used for testing. It was ascertained the impact of encapsulated and non-encapsulated thyme essential oil on tested microorganisms.

Keywords*: Thyme, Essential Oil, Encapsulated Essential Oil, Polyethylene Foil, Antimicrobial Activity*

Abbreviations: EO – essential oil

INTRODUCTION

Some plants are rich in functional compounds as phenols, polyphenols, micronutrients or essential oils [1]. Different biological activities (as antioxidant, antifungal and antibacterial activities) were demonstrated for these compounds. Phenols influence enzyme activity, cause protein denaturation and cell membrane damages of its function or structure, which lead to loss of macromolecules [2]. Polyphenolic compounds have strong antimicrobial activity, which consist in modification of the morphology and disruption of the cell wall. Other phenolic compounds can stimulate DNA degradation [3].

Thyme (*Thymus vulgaris*, L.) belongs to plants with strong antimicrobial activity. The majority of *Thymus* oils are characterized by their rich content of monoterpenes, in particular the phenolic compounds thymol and its isomer carvacrol, accompanied by a range of other more or less biologically active compounds, including eugenol, p-cymen, γ-terpinen, α-pinen, linalool, geraniol and borneol [4,5]. Antimicrobial activity of thyme essential oil was demonstrated in a lot of studies. Thyme suppresses growth of *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Escherichia coli* and *Candida* sp. [5,6]. Thyme essential oil has significant colicid and colistatic properties and damages irreversible the *E. coli* O157:H7 cells within one minute [1,7].

In this study, the antimicrobial effect of encapsulated and non-encapsulated essential oil was tested against selected bacterium, yeast and mould.

EXPERIMENTAL

Microorganisms, which were used for analyses, were *Escherichia coli* CCM 7929, *Candida tropicalis* CCM 8223 and *Penicillium chrysogenum* CCM 8034. 24 hours culture of *E. coli*, which was cultivated in TSB (Biokar Diagnostics, France) at 37 °C, was centrifuged (20 min., 3000 rpm), rinsed with saline and once more centrifuged (20 min., 3000 rpm). A solution of density 1 McF was prepared, which was diluted to density 0.1 McF. 72 hours culture of *Candida tropicalis* and 120 hours culture of *Penicillium chrysogenum*, which were cultivated on Chloramphenicol Glucose Agar (Biokar Diagnostics, France) at 25 °C, were transferred into wells with sterile saline and carefully mixed. Then the solution of density 1 McF was prepared. Solution was diluted to density 0.1 McF.

Sliced cheese (Albert Quality 30 % TVS) was used for analyses with non-encapsulated thyme

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essential oil. One slice was divided into quarters, which were placed in Petri dishes and exposed to UV radiation for 45 minutes. Non-encapsulated thyme essential oil (Manipura, Czech Republic) was used undiluted (concentration 1) and diluted by adding 6 µl into 1 ml of methanol (concentration 2). The sterile paper disc (diameter 9 mm) was saturated with 30 µl of essential oil of appropriate concentration. Disc saturated with methanol was used as a control.

Polyethylene foil, which is coated with partially water soluble polymeric film containing encapsulated thyme essential oil, was used for analyses with encapsulated thyme EO. Samples of this foil with different concentration of EO (variants 1 – 5) were divided into 2x2 cm squares and exposed to UV radiation for 45 minutes. Concentrations of EO are stated in Table 1.

Table 1 Concentration of thyme EO on polyethylene foil

Variant	Weight, %
1	1.6
2	3.5
3	5.8
4	8.6
5	10.3

Volume 0.1 ml of microbial culture of density 0.1 McF was inoculated on VRBL Agar (*E. coli*), on Chloramphenicol Glucose Agar (*C. tropicalis*, *P. chrysogenum*) or on cheese. Squares of tested foil or discs with EO were attached on the inside part of lids of Petri dishes by double-faced tape. In the second variant, squares of foil were placed directly on the surface of nutrient medium. All these variants were prepared in triplicate. Petri dishes were incubated at 37 °C for 24 hours (*E. coli*), at 25 °C for 24 (*C. tropicalis*) or 48 hours (*P. chrysogenum*). After cultivation, the diameters of inhibitory zones were evaluated with a ruler.

RESULTS

There are not any visible changes after 24 hours on slices of cheese when testing thyme essential oil efficacy against *E. coli*. Any zones of inhibition were not observed in Petri dishes with foil without direct contact. In the case of *C. tropicalis* and *P. chrysogenum*, there were not created clear zones of inhibition. The evaluation was therefore reduced on appraisal of growth of yeasts or moulds on the whole cheese surface. Average sizes of diameters of inhibitory zones or intensity of microbial growth are shown in Tables 2, 3 and 4.

Concentrated essential oil had considerable inhibitory effect on *E. coli* on nutrient medium. Encapsulated essential oil was effective at all concentrations with increasing tendency with increasing concentration.

Candida tropicalis was inhibited almost by all EO concentrations in all variants of experiment. Variants of foil 1 and 2 were the exceptions, where there was no reduction in growth of *C. tropicalis*. It was observed an increase in diameters of inhibitory zones or in growth intensity with increasing concentration of essential oil.

Non-encapsulated EO was effective in concentrated form, diluted EO showed no antifungal activity on cheese surface. On nutrient medium, there was significant difference between efficacy of diluted and concentrated EO. Encapsulated EO inhibited growth of *P. chrysogenum* by direct contact in all variants. Essential oil without direct contact suppressed growth of *Penicillium* only in variants 4 and 5, so only at higher concentrations.

Table 2. Impact of thyme EO on *E. coli*

Sample	EO	Encapsulation	Culture Medium	Diameter [mm]
1	Conc.	No	Nutrient Medium	24.67
2	Diluted			0.00
3	Control			0.00
4	V. 1	Yes	Nutrient medium Direct contact	24.33
5	V. 2			30.00
6	V. 3			36.67
7	V. 4			50.00
8	V. 5			48.33

V. 1 – V. 5 – Variants of polyethylene foil with various concentrations of thyme EO
Conc. – Concentrated EO
Diameter – Average size of the identified zones of inhibition

Table 3. Impact of thyme EO on *C. tropicalis*

Sample	EO	Encapsulation	Culture Medium	Growth Intensity / Diameter [mm]
9	Conc.	No	Cheese	+
10	Diluted			++
11	Control			+++
12	Conc.	No	Nutrient Medium	38.33
13	Diluted			10.00
14	Control			0.00
15	V. 1	Yes	Nutrient Medium Direct Contact	20.00
16	V. 2			23.67
17	V. 3			30.00
18	V. 4			35.00
19	V. 5			35.33
20	V. 1	Yes	Nutrient Medium Without Direct Contact	0.00
21	V. 2			0.00
22	V. 3			10.67
23	V. 4			35.00
24	V. 5			36.67

+ Low growth intensity
++ Medium growth intensity
+++ High growth intensity

Table 4. Impact of thyme EO on *P. chrysogenum*

Sample	EO	Encapsulation	Culture Medium	Growth Intensity / Diameter [mm]
25	Conc.	No	Cheese	+
26	Diluted			+++
27	Control			+++
28	Conc.	No	Nutrient Medium	53.33
29	Diluted			18.33
30	Control			0.00
31	V. 1	Yes	Nutrient Medium Direct Contact	20.00
32	V. 2			20.00
33	V. 3			20.00
34	V. 4			27.67
35	V. 5			26.00
36	V. 1	Yes	Nutrient Medium Without Direct Contact	0.00
37	V. 2			0.00
38	V. 3			0.00
39	V. 4			20.00
40	V. 5			20.00

DISCUSSION

Susceptibility of tested microorganisms was dissimilar. In experiment with cheese, *C. tropicalis* was inhibited by EO at most. Reduce in growth was observed even by application of diluted essential oil. Important antifungal activity was proved in [5,8,9]. Non-encapsulated EO was more effective when microorganisms were cultivated on nutrient medium than on cheese. In this case, bigger zones of inhibition were observed. *P. chrysogenum* was the most susceptible; *E. coli* was inhibited at least. All the tested microorganisms were suppressed by encapsulated EO. The greatest diameters of

inhibitory zones were detected in direct contact of encapsulated essential oil with microorganisms. *E. coli* was the most susceptible to the direct contact with EO, inhibition zones measured from 22 to 50 mm. Burt and Reinders (2003) [7] and Imelouane et al. (2009) [10] ascertained that thyme inhibits wide range of microorganisms, including *E. coli*. *P. chrysogenum* was not so much repressed. Inhibition zones were from 20 to 30 mm. Encapsulated EO without direct contact was less effective. Antimicrobial activity was not proved against *E. coli*, there were no inhibitory zones. *P. chrysogenum* was susceptible to EO effects only at higher concentrations (variant 4 and 5). Size of inhibitory zone was 20 mm at both concentrations. *C. tropicalis* was susceptible to EO at variants 3, 4 and 5.

CONCLUSIONS

Thyme EO repressed all the tested microorganisms with different potency. Undiluted essential oil was naturally more effective than diluted EO, but even diluted EO inhibited visibly some tested microorganisms. Encapsulated EO had more considerable activity in direct contact with nutrient medium. The present results indicate that thyme has provable antimicrobial activity and could be used for repression of undesirable microorganisms and their elimination from food.

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АНТИМИКРОБИАЛЕН ЕФЕКТ НА ИНКАПСУЛИРАНО И НЕ-ИНКАПСУЛИРАНО ЕТЕРИЧНО МАСЛО ОТ МАЩЕРКА

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(Резюме)

Някои растения съдържат функционални компоненти с антимикробиална активност. Към тях спадат феноли, полифеноли, микроелементи и етерични масла. Биоактивните компоненти могат да се използват срещу патогени и бактерии, които водят до разваляне на храната. Етеричното масло от мащерка принадлежи към растителните продукти със силна антимикробиална активност. Целта на работата беше да се тества антимикробиалната активност на етерично масло от мащерка и полиетиленово фолио, което е покрито с частично водоразтворим полимерен филм, съдържащ инкапсулирано етерично масло от мащерка, върху подбрани микроорганизми при и без директен контакт. За тестването бяха използвани Еширихия коли (*Escherichia coli*), Кандида тропикалис (*Candida tropicalis*) и Пеницилиум хрисогениум (*Penicillium chrysogenum*). Констатирано е въздействието на инкапсулирано и неинкапсулирано етерично масло от мащерка върху тестваните микроорганизми.