STUDYING THE POSSIBILITIES OF USING OF ESSENTIAL OILS IN DAIRY PRODUCTS. OREGANO (ORIGANUM VULGARE L.)

***[1]Kostova I.**
[1]Dimitrov D., Ivanova M., [2]Vlaseva R., Damyanova S., Ivanova N., Stoyanova A.

[1]Ruse University „A. Kanchev", Branch–Razgrad
[2]University of food technologies – Plovdiv
***Kostova I., e–mail: ikostova@uni–ruse.bg**

Abstract: The possibility of using of the essential oil of oregano *(Origanum vulgare L.)* in dairy products has been studied. The composition, antimicrobial properties and its effect on the microorganisms of starter cultures for dairy products has been studied. It was found that it exhibits high microbial activity, but does not inhibit the development of the lactic acid bacteria in dairy starter cultures. The essential oil of oregano is a suitable natural addition to dairy products.

Keywords: oregano, essential oil, antimicrobial properties, microorganisms, lactic acid bacteria

Introduction

Oregano (*Origanum vulgare L.*) is a perennial herbaceous plant of the family Lipped (*Lamiaceae*). It grows in stony places and rare woods primarily in foothill and mountain areas up to 1600 m altitude. Oregano is known since ancient times and is used as a spice and herb. It is a rich source of minerals – sodium, calcium, iron, and manganese. It has a pronounced anti– inflammatory, antibacterial, antioxidant, and tonic effect [3, 4, 8, 9]. Oregano contains essential oil, provitamin A, flavonoids, tannins, bitter compounds, ascorbic acid, tricyclic sesquiterpenes, etc, that makes it applicable in food, cosmetics and pharmaceuticals. The chemical composition of the oil depends on the origin of the plant and the soil on which it is grown. It is rich in phenols (thymol and carvacrol), there is little borneol, pinene, and terpineol and traces of esters. Thymol and carvacrol have proven antibacterial effect including against common inflections caused by streptococcal bacteria. Essential oil helps with indigestion, balances, soothes. It has analgestic and blood pressure lowering effect. It is used in cosmetics – shampoos, conditioners, lotions, emulsions and massage, baths, inhalations, and compresses too.

Since oregano has strong flavor and facilities digestion, it occupies an important place in Italian, Greek, Swiss, and others cuisines using for flavoring and seasoning pizza, tomatoes, cheese, vegetable, fish, and meat dishes. There is no data in the literature for using the oregano oil in food products [8, 13, 17, 18] .

The purpose of this work is to explore the possibilities for using of essential oil of oregano *(Origanum vulgare L.)* in dairy products by examining the composition, antimicrobial properties, and its effect on microorganisms by starter cultures for dairy products.

Experimental

Materials: Oil of oregano *(Origanum vulgare L.)* has been used, provided by the company Vigalex Ltd, Sofia. Test microorganisms: To determine the antimicrobial activity of the oil of oregano are used test cultures from NBIMCC – National Bank of Industrial Microorganisms and Cell Cultures, Sofia: Gram–positive: *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis ATCC 6633;* Gram–negative: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa ATCC 9027, Salmonella abony* NTCC 6017; Yeasts: *Saccharomyces cerevisiae* ATCC 9763, *Candida albicans* ATCC 10231; Fungi: *Aspergillus niger* ATCC 16404, *Penicillium chrysogenum, Fusarium moniliforme.* Starter cultures: Two starter cultures have been used for white brine cheese: "MikroMILK TBMC1", contains: *Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactococcus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus casei* and „LB Bulgaricum JSC LBB CM 310–40" with composition: *Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus, Lactobacillus casei, Lactobacillus helveticus, Lactococcus lactis.*

Methods: *Determination of the composition of the essential oil of oregano:*

The chemical composition of the oil was determined chromatographically. For GC analysis was used apparatus: GC 7890 A with MSD 5795 C;Temperature program 40 °C for 3 min then 5 °C/min to 300 °C for 5 min, run time 60 min; Column: HP–5MS (30 m x 250 µm x 0.25 µm); Gas: helium with a flow rate of 1 cm^3/min.

Determination of the antimicrobial activity of oil of oregano by agar diffusion method: The experiments were conducted on Tryptic Soy agar (Biolife) – for bacterias and Sabourad– dextrose agar (Biolife) for the yeasts and fungi. The oil of oregano was tested at concentrations of 100; 50; 10; 5; 1; 0,5; 0,1; 0,05 % Tween 80 in solution.

The diameters of the zones of the growth inhibition were measured in mm with a digital caliper, such as up to 15 mm microbial culture is less sensitive; from 15 to 25 mm – sensitive and over 25 mm – highly sensitive. The experiments were conducted in parallel with controls from the solvent, taking into account and correct its effect.

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): MIC and MBC were determined according to the methods described by Andrews [1], Barros et al. [2] and Smith–Palmer et al. [16].

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the oil of oregano on the microorganisms in starter cultures: The following concentrations of the oil were prepared: 1; 0,5; 0,1; 0,05 and 0,01 % in solvent sterile 1 % solution of Tween 80. The growth of the bacterial cultures was assessed by comparing the number of the lactic acid bacterial in each suspension with that one of the control (bacteria suspension without oil). Inhibition of the growth of the lactic acid bacteria is read at that concentration of the essential oil in which is established 90 % reduction in the number of lactic acid bacteria compared to the control samples.

Studying of the influence of the oil of oregano at concentrations suitable for the addition in food products on the microorganisms of the starter cultures: The studying is conducted according to the procedures for determine to the total number of lactic acid bacteria in starter cultures [15].

All results are presented as average of three parallel experiments.

Results and discussion

Table 1 presents the chemical composition of the essential oil. Data show that here contained 44 components (85,2% identified). Three of them are in a quantity exceeding 3 % and 41 below 3 % : carvacrol (62,14 %), p–cymene (10,31 %) and γ–terpinene (6,06 %). The content of the basic components of the oil does not differ significantly from the data in the literature, the difference is due to the different origin of the raw material, soil, climatic conditions, and method of storage [8, 5, 11, 14, 18].

Table 1 Chemical composition of the essential oil of oregano

Components	*RI*	*Quantity, %*	*Components*	*RI*	*Quantity, %*
α–Thujene	929	1,16	***Bornyl acetate***	1285	0,15
α–Pinene	939	0,96	***Thymol***	1290	0,26
Camphene	954	0,37	***Carvacrol***	1298	62,14
Sabinene	975	0,29	***α–Terpinyl acetate***	1348	0,11
β–Pinene	979	0,41	***α–Copaene***	1377	0,09
Octen–3–ol	982	0,09	***α–Cubebene***	1385	0,26
α–Phelandrene	1003	0,11	***β–Caryophyllene***	1419	0,27
α–Terpinene	1018	1,63	***β–Cubebene***	1429	2,12
p–Cymene	1025	10,31	***β–Gurjunene***	1432	0,47
α–Limonene	1030	2,38	***Aromadendrene***	1441	0,23
Eucalyptol	1034	0,08	***α–Humulene***	1454	0,07
(Z)–β–Ocimene	1038	0,11	***allo–Aromadendrene***	1461	0,28
(E)–β–Ocimene	1048	0,14	***γ–Muurulene***	1478	0,36
γ–Terpinene	1060	6,06	***Germacrene D***	1485	0,08
cis–Sabinene hydrate	1070	0,47	***α–Muurolene***	1501	0,11
Terpinolene	1088	0,23	***β–Bisabolene***	1509	0,09
trans–Sabinene hydrate	1097	0,09	***γ–Cadinene***	1514	0,13
β–Linalool	1099	0,23	***δ–Cadinene***	1523	0,15
Terpinen–4–ol	1177	1,87	***α–Cadinene***	1539	0,06
α–Terpineol	1188	0,16	***Spathulenol***	1578	0,10
Methyl thymyl ether	1215	0,21	***Caryophyllene oxide***	1580	0,12
Carvone	1248	0,33	***α–Cadinol***	1640	0,17

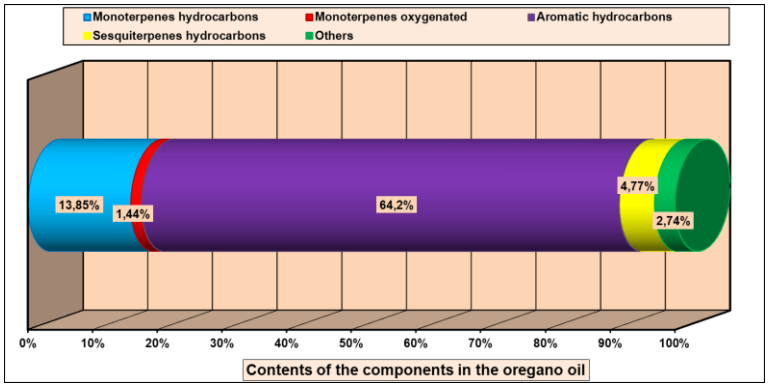

Fig.1. Distribution of the components of the oregano oil

Figure 1 shows the distribution of the components of the oil groups. The data shows that the aromatic oxygen compounds (62,4 %), predominate, followed by monoterpenes hydrocarbons (13,85%). Significantly lower is the amount of sesquiterpenes and monoterpenes oxygen compounds.

The results of the antimicrobial activity of the oil of oregano are shown in Figure 2 and 3.

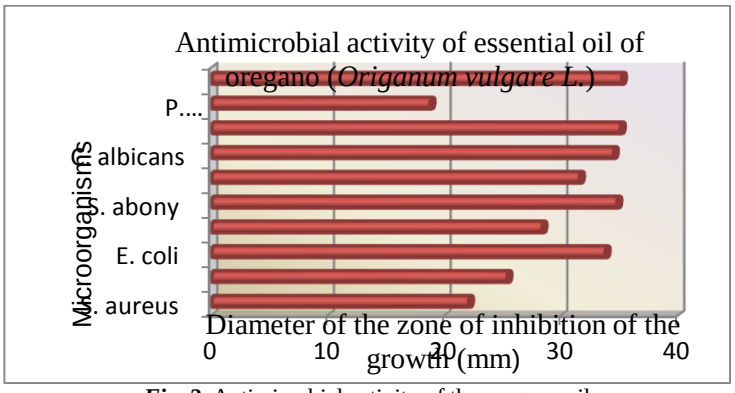

Fig. 2. Antimicrobial activity of the oregano oil

Tested Gram–positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* are sensitive to the essential oil of oregano. More pronounced is the antibacterial activity against *Bacillus subtilis* (25,4mm zone diameter).

The presented data shows that the oil of oregano has strong antimicrobial activity against Gram–negative bacteria. Of these *Escherichia coli* and *Salmonella abony* are more pronounced sensitivity (34,8 mm and 33,8 mm diameter area, respectively) compared to P*seudomonas aeruginosa*. The results show that the oil of oregano has stronger antimicrobial activity against Gram– negative bacteria than Gram – positive.

The yeasts *Saccharomyces cerevisiae* and *Candida albicans* and the fungi *Aspergillus niger*, *Penicillium chrysogenum* and *Fusarium moniliforme* also strongly

inhibited by the oil of oregano. Antifungal activity of the oil is less pronounced against *Penicillium chrysogenum* (18,8 mm zone diameter), while others are highly sensitive to its effect (up 30 mm diameter of the zone of the growth inhibition).

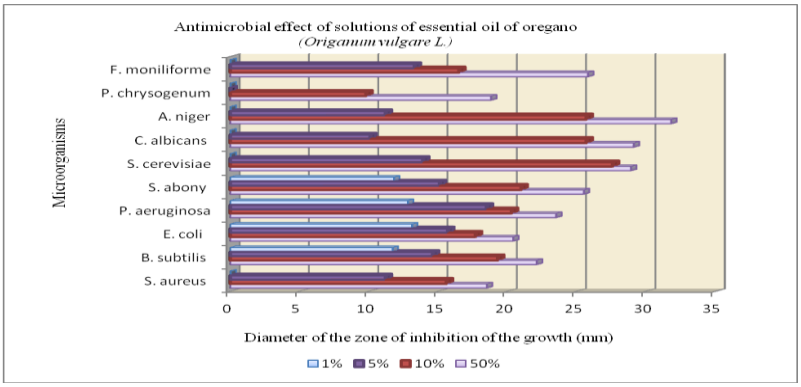

Fig. 3. Antimicrobial effect of 50, 10, 5 and 1% solutions of the oregano oil

The presented in Figure 3 results show that the tested Gram– positive, Gram–negative bacteria, yeasts and fungi are sensitive to 50 and 10% solutions of the essential oil of oregano (*Origanum vulgare L.*). The zones of the growth inhibition are greatest in *Salmonella abony*, *Saccharomyces cerevisiae*, *Candida albicans* and *Aspergillus niger*. With the exception of *Penicillium chrysogenum* all test microorganisms are sensitive although more weakly to 5% solution of the oil of oregano. Gram–positive bacteria *Staphylococcus aureus* and all tested yeasts and fungi are resistant to 1% solution of the oil.

Since the essential oil of oregano (*Origanum vulgare L.*) has antibacterial activity and 1% solutions, to determine the minimum inhibitory (MIC) and minimum bactericidal concentration (MBC) experiments were made with solution of the oil in concentrations of 1; 0,5; 0,1; 0,05%.

Table 2 shows that the minimum inhibitory concentration (MIC) of the oil of oregano is 0,1%, and the minimum bactericidal concentration (MBC) – 0,5%. The antimicrobial activity of the oil of oregano analyzed by us is higher than in the literature [2, 7, 10, 11].

To be used in dairy products the essential oil should not inhibit the development of the lactic acid bacteria contained in the dairy starter cultures. In this connection, the influence on the lactic acid bacteria from the two starter cultures is analyzed. The obtained results are shown in Table 3.

The data shows that the growth of three kinds *Lactobacillus*, participating in the composition of the two starter cultures is not affected by the 0,01 % solution of the essential oil. 0,05 % solution of the oil substantially inhibit the growth of lactobacilli. The number of viable Colony Forming Units decreases strongly with both starter cultures. By increasing the concentration of the oil over 0, 5% it does not observe growth of *Lactobacillus sp.* Therefore the MIC of the oil of the oil of oregano against *Lactobacillus sp.* is 0,05 %, and MBC is 0,5%.

Table 2. Effect of solutions of essential oil of oregano on the growth of test microorganisms

Test microorganisms	***Total number of viable microorganisms (cfu/ml)***				
	control	***Oregano– oil concentration***			
		0,05%	***0,1%***	***0,5%***	***1,0%***
Staphylococcus aureus ATCC 6538	$5,1x10^8$	$3,1x10^8$	$1,6x10^4$	0	0
Bacillus subtilis ATCC 6633	$2,2x10^8$	$3,0x10^8$	$2,0x10^4$	0	0
Escherichia coli ATCC 8739	$6,2x10^8$	$3,9x10^8$	$4,7x10^4$	0	0
Pseudomonas aeruginosa ATCC 9027	$3,0x10^8$	$1,1x10^8$	$1,9x10^4$	0	0
Salmonela abony NCTC 6017	$1,6x10^8$	$1,3x10^8$	$2,3x10^6$	0	0

Table 3. Effect of solutions of essential oil of oregano on the growth of lactic acid bacteria from starters for dairy products

Essential oil	Concentration (μg/ml)	Total number of viable *Lactobacillus sp* (cfu/ml)		Total number of viable *S. thermophilus, L. lactis* (cfu/ml)	
		Starter LBB CM 310–40	Starter TBMC1	Starter LBB CM 310–40	Starter TBMC1
Control	0	$3,1x10^7$	$2,8\ x10^7$	$1,4x10^8$	$2,4x10^8$
Oregano	0,01	$1,6x10^7$	$1,1\ x10^7$	$2,3x10^7$	$1,7x10^7$
	0,05	$9,1x10^4$	$8,0\ x10^4$	0	0
	0,5	0	0	0	0
	1,0	0	0	0	0

The table also shows that the oil of oregano affects the growth of *S. thermophilus and L. lactis* – 0,01% solution inhibits their development, over 0,05% completely killing them. Therefore MIC of the oregano oil against *S. thermophilus and L. lactis* e 0,01%, and MBC is 0,05 %.

The addition of essential oils in food products requires an appropriate amount so as not to deteriorate their quality. On the other hand, they must not have depressing effect on the specific microorganisms, involved in the preparation of the fermented milk product. I n this connection, the influence of the oregano oil is analyzed at concentrations 0,0008, 0,002 and 0,003 %, which can be used in food products [8, 12]. The obtained results are shown in Table 4.

Table 4. Effect of the essential oil of oregano on lactic acid bacteria from starters for dairy products

Essential oil	***Concentration (%)***	***Total number of viable***		***Total number of viable S. thermophilus, L. Lactis (cfu/ml)***	
		LBB CM 310–40	***TBMC1***	***LBB CM 310–40***	***TBMC1***
Control		$3,3x10^7$	$2,8x10^7$	$2,3x10^8$	$2,5x10^8$
Oregano	***0,0008***	$3,2x10^7$	$5,1x10^7$	$2,6x10^8$	$3,1x10^8$
	0,002	$2,7x10^7$	$1,2x10^7$	$3,1x10^8$	$2,9x10^8$
	0,003	$2,1x10^7$	$3,8x10^7$	$3,3x10^8$	$3,4x10^8$

The growth of *Lactobacillus* in the two starter cultures used is not affected by the presence of the essential oil of oregano in concentrations which can be used in food products. The number of Colony Forming Units with the addition of essential oil is approximately equal to the number of the colonies in the control samples. *S. thermophilus and L. lactis* is also not sensitive to the used concentrations of the oregano oil in the tested starters: LBB CM 310–40 and TBMC1.

Our results confirm those in the literature [6, 12].

Conclusion

The essential oil of oregano exhibits high antimicrobial activity but does not inhibit the development of the lactic acid bacteria in dairy starter cultures. Minimum inhibitory concentration of the oregano oil against *Lactobacillus sp.* is 0,05 %, compared to *Streptococcus thermophilus* and *Lactococcus lactis* – 0,01 %. Minimum bactericidal concentration against *Lactobacillus sp* is 0,5 %, compared to *Streptococcus thermophilus and Lactococcus lactis* is 0,05%. The essential oil of oregano is suitable natural addition to dairy products.

References

1. Andrews J. M. – Determination of minimum inhibitory concentrations, Journal of Antimicrobial Chemotherapy, 48, Suppl. S1, 2001, 5–16.

2. Barros C., M. da Conceição, N. Gomes, A. da Costa, E. da Souza – Combination of *Origanum vulgare* L. essential oil and lactic acid to inhibit *Staphylococcus aureus* in meat broth and meat model, Brazilian Journal of Microbiology, 2012, 1120 – 1127.

3. Celikel N., G. Kavas – Antimicrobial properties of some essential oils against some pathogenic microorganisms, Czech J. Food Sci. 26, 2008, 174–181.

4. Cleff M. B., A. R. Meinerz, M. Xavier, L. F. Schuch, M. C. A. Meireles, M. R. A. Rodrigues, J. R. B. Mello – *In vitro* activity of *Origanum vulgare* essential oil against *Candida* species, Brazilian Journal of Microbiology 2010, 41: 116–123.

5. Figiel A. – Composition of oregano essential oil (Origanum vulgare) as affected by drying method, Journal of Food Engineering 2010, 98: 240–247.

6. Gutierrez J., C. Barry–Ryan, P. Bourke – Antimicrobial activity of plant essential oils using food model media: efficacy, synergistic potential and interaction with food components, Food Microbiology, Vol. 26, issue 2, April 2009, 142–150.

7. Hammer A., C. Carson, T. Riley – Antimicrobial activity of essential oils and other

plant extracts, Journal of Applied Microbiology, 86, 1999, 985–990.

8. Hristov N.– Bulgarska enciklopediya na eterichnite masla, Pleven 2008.

9. Ivanov I., I. Landjev, G. Neshev – Bilkite v Bulgaria i zpolzvaneto im, Zemizdat, 1977.

10. Kačániová M., N. Vukovič, L. Hleba, A. Bobková, A. Pavelková, K. Rovná, H. Arpášová – Antimicrobial and antiradicals activity of Origanum vulgare L. and Thymus vulgaris essential oils, Journal of Microbiology, Biotechnology and Food Sciences, 2012: 2 (1) 263–271.

11. Mayaud L., A. Carricajo, A. Zhiri, G. Aubert – Comparison of bacteriostatic and bactericidal activity of 13 essential oils against strains with varying sensitivity to antibiotics, Letters in Applied Microbiology 47, 2008, 167–173.

12. Olmedo H., V. Nepole, N. Grosso – Preservation of sensory and chemical properties in flavoured chees prepared with cream cheese base using oregano and rosemary essential oils, LWT – Food Science and Technology, 2013, 53, pp 409 – 417.

13. Petrovski S., S. Stoyanov – Eterichni masla I prilojenieto im v medicinata I prmishlenosta, Publish Site Set–Eco, Sofia, 2005.

14. Raduðienë J., A. Judþintienë, D. Peèiulytë, V. Janulis – Chemical composition of essential oil and antimicrobial activity of Origanum vulgare, Biologija, 2005, 4. p. 53–58.

15.Slavchev G., R. Enikova, M. Makaveeva – Rakovodstvo za fizikoximichen I microbiologichen kontrol na mlyako I mlechni produkti, Asociaciya na mlekoprerabotvatelite v Bulgaria, Sofia, 2000.

16. Smith–Palmer A., J. Stewart, L. Fyfe – Antimicrobial properties of plant essential oils and essences against five important food–born pathogens, Letters in Applied Microbiology, 1998, 26, 118 – 122.

17. Souza E. L., T. L. M. Stamford, E. O. Lima – Sensitivity of spoiling and pathogen food–related bacteria to *Origanum vulgare L.* (Lamiaceae) essential oil, Brazilian Journal of Microbiology 2006, 37: 527–532.

18. Teixeira B. – Chemical composition and bioactivity of different oregano (Origanum vulgare) extracts and essential oil, J Sci Food Agric (Portugal) 2013, 93: 2707–2714.