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ANTIMICROBIAL ACTIVITY OF VARIOUS ESSENTIAL OILS AGAINST SPOILAGE AND PATHOGENIC MICROORGANISMS ISOLATED FROM MEAT PRODUCTS

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ABSTRACT

Antimicrobial activity of essential oils from Origanum vulgare, Satureja montana, Thymus vulgaris, Pimenta dioica and its major constituents against spoilage and pathogenic microorganisms isolated from meat products was evaluated. Among used test microorganisms Pseudomonas aeruginosa demonstrated the highest resistance and Staphylococcus aureus demonstrated the highest susceptibility to all of the studied essential oils. It was determined that antimicrobial activity of essential oils depended mainly on total content of carvacrol, thymol and eugenol in oil samples. Antimicrobial activity of essential oils decreased parallel to decreasing of carvacrol, thymol and eugenol content. The highest activity demonstrated the oil from O. vulgare, followed by P. dioica, S. montana and T. vulgaris. The studied essential oils from spices were prospective natural antimicrobial for application in food industry for partial replacement of synthetic antimicrobials in meat products.

Keywords: antimicrobial activity, essential oils, meat products, spoilage bacteria

Introduction

The attitude of people to food, as natural and inevitable necessity, has altered in line with the change of social conditions and development of society. Undoubtedly this is proof of the huge interest in current consumers in Europe and the world, to show the composition, properties, safety and health effect of food products which consume. The desire to consume foods with high biological value, natural origin and composition, with a long shelf life, poses a huge challenge for modern food science and industry in all directions. To meet these requirements, European Agency for Food Safety (EFSA) directs about 100 billion Euros for researches in several major scientific fields related to development, application and synchronization of systems for food safety monitoring and control in the EU. As recommended by the World Health Organization (WHO), intensive search for natural food preservatives, for partial or complete replacement of currently applied synthetic antimicrobials in foods are carried out. To find real practical application in food industry, natural antimicrobials must be harmless and posses specific mechanism of antimicrobial action even at low concentrations and to have no effect on organoleptic properties and biological value of foods. Essential oils from various spices fulfill the above listed requirements to a highest extend. The flavor and medicinal power of essential oils from spices have been utilized by humanity for millennia. Currently essential oils isolated from the aerial parts of spices, belonging to the genera *Thymus, Origanum* and *Satureja* (*Lamiaceae*) are widely applied in food-flavoring, pharmaceutical industry and cosmetics (12-14,19). Many papers discussed antimicrobial activity of oregano oil (*Origanum vulgare*), savory oil (*Satureja montana*), thyme oil (*Thymus vulgaris*) and pimento oil (*Pimenta dioica*) (1,3-5,14-18,20). It is very difficult to compare the published results, because different techniques for antimicrobial testing are applied and different test microorganisms are used (11). Usually reference strains are used for evaluating of antimicrobial activity of the essential oils, but from practical point of view it is very important to test essential oils against microorganisms isolated from spoiled foods.

The aim of this study is to evaluate antimicrobial activity of thyme oil, savory oil, oregano oil and pimento oil against various microorganisms isolated from meat products. Preliminary screening of effective essential oils was carried out by disc diffusion method. MIC and MBC of the oils were

also determined.

Materials and methods

Essential oil samples and reference compounds

The essential oils of oregano (O*riganum vulgare* L), savory (*Satureja montana*, L), thyme (*Thymus vulgaris*, L), pimento (*Pimenta dioica, L*), carvacrol, thymol and eugenol were purchased from Kurt Kitzing Co., Wallerstein, Germany. Chemical composition of the essential oils was determined by GC/FID and CG/MS and published earlier (4).

Test microorganisms and preparation of test inoculum

Staphylococcus aureus, *Escherichia coli*, *Citrobacter diversus, Enterobacter amnigenus, Proteus vulgaris, Providencia rettgeri, Pseudomonas aeruginosa* and *Candida albicans* were isolated from meat products and identified to species level at Dep. "Biochemistry and microbiology" at University of Plovdiv and deposited in department culture collection. Bacteria were maintained on Nutritional Agar (NA), National Center of Infectious and Parasitic Diseases (NCIPD, Sofia, Bulgaria). Overnight bacteria cultures were prepared by inoculating about 2 mL of Mueller-Hinton Broth (MHB, NCIPD) with 2-3 colonies selected from NA. Broths were incubated at 37ºC for 24 h on a rotary shaker 220 rev/min. Inoculums were prepared by diluting overnight cultures by adding sterile MHB to achieve absorbance, corresponding to 0.5 McFarland turbidity standard. The yeast inoculum was prepared on the same way, but Sabouraud Agar (SA) and Sabouraud Broth (SA, SB, and NCPID) were used.

Disc diffusion method

Disc diffusion method was carried out as described by Sacchetti et al. (15) in accordance with NCCLS recommendations (9). The Petri dishes (d= 90mm), containing solidified MHA or SA were inoculated by spreading 100µL of the inoculum. Sterile paper discs Whatman 1 (d= 5mm, NCIPD) were soaked with 10µL of undiluted essential oil and placed on the inoculated surface of Petri dishes. Petri dishes were incubated at 37ºC for 24h. The growth inhibition zone diameter (IZ, mm) was measured to the nearest millimeter. Each experiment was performed in duplicate.

Serial broth dilution method

Serial broth dilution method was carried out in accordance with NCCLS recommendations (8,10). A stock solution to be tested was prepared by diluting peppermint oil sample in DMSO (Sigma-Aldrich Co.). Stock solution was then added to culture broth to reach final oil concentrations ranging from 3.28% (v/v) to 0.01% (v/v). Serial dilutions were inoculated with 100µL of bacteria inoculum, prepared as listed above. The samples were then incubated at 37ºC for 24h and the absorbance was read at 680 nm Control samples of inoculated broth without oil and without DMSO and inoculated broth with DMSO, were also incubated under the same conditions. The concentration of DMSO in the broth dilution assay was kept at defined concentration to ensure that the effect on bacterial and yeast growth was minimal. Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration which resulted in a reduction of > 90% in the observed absorbance (). To determine Minimal Bactericidal Concentration (MBC) and Minimal Fungicidal Concentration (MFC), 100µL of each dilution showing no growth was spread on MHA and SA, respectively. The inoculated Petri dishes were incubated at 37ºC for 24h. The colony forming units were counted and compared to control dishes. MBC and MFC were defined as the lowest concentration that killed > 99.9% of the initial inoculum (11). Each experiment was performed in duplicate.

Results and Discussion

It is well known that antimicrobial activity of essential oils depends mainly on its chemical composition (2,4,7). Chemical composition of the studied essential oils was evaluated earlier and thymol was determined as the major constituent of thyme oil - 43.4% (4). Carvacrol was the major constituent of savory oil and oregano oil, 41.5% and 66%, respectively (4). Eugenol was the major constituent of pimento oil – 76.02% (4). Agar disc diffusion method is recommended for preliminary testing of antimicrobial activity of natural compounds (11). For this reason the studied essential oils and its major constituents was tested firstly by disc diffusion method. The results are shown in **Table 1**.

As seen all of the investigated essential oils, thymol, carvacrol and eugenol demonstrated antimicrobial activity against all of the used test microorganisms. MIC, MBC and MFC of the oils and the pure compounds were determined by broth dilution method, which is more reliable and repeatable than disc diffusion method (11,14). The results are shown in **Table 2**.

As seen the essential oil of oregano demonstrated the highest antimicrobial activity, followed by pimento oil, savory oil and thyme oil. *P. aeruginosa* demonstrated the

highest resistance against all of the investigated essential oils and *S. aureus* demonstrated the highest susceptibility. Gram-negative bacteria were more resistible comparing to Gram-positive, which can be attributed to differences in cell wall composition and architecture (1-4).

TABLE 1

Zones of growth inhibition of investigated essential oils and major constituents

Test microorganism	IZ, mm						
	T.vulgaris	*S.montana*	*O.vulgare*	*P.dioica*	*Thymol*	*Carvacrol*	*Eugenol*
S.aureus	36.3	37.5	45.3	41.7	33	32.8	35.5
E.coli	33.3	34.3	36.3	35.8	32.2	32	32.3
C.diversus	34.5	36.5	38.3	37.5	33	32.5	34.5
E.amnigenus	18	19.5	22.8	21.7	17	17	17.7
P.mirabilis	32.7	33.8	35.3	34.8	31	31.3	32
P.rettgeri	26.5	28.2	30.8	29.5	24.3	24	24.8
P.aeruginosa	14.8	16	17.8	16.8	13	13	14.7
C.albicans	32.3	35	35	34.5	32	32	36.3

TABLE 2

MIC, MBC and MFC of investigated essential oils and major constituents.

Test microorganism	MIC/MBC*, % (v/v)						
	T.vulgaris	*S.montana*	*O.vulgare*	*P.dioica*	*Thymol*	*Carvacrol*	*Eugenol*
S.aureus	0.1/0.2	0.05/0.1	0.05	0.05/0.1	0.2/0.4	0.2/0.4	0.2
E.coli	0.4	0.2/0.4	0.1/0.2	0.2/0.4	0.4	0.4	0.2/0.4
C.diversus	0.2	0.1/0.2	0.1/0.1	0.1/0.2	0.2/0.4	0.2/0.4	0.2
E.amnigenus	0.8/0.8	0.4/0.8	0.2/0.4	0.4/0.8	0.8/1	0.8/1	0.8
P.mirabilis	0.4/0.8	0.2/0.4	0.1/0.2	0.2/0.4	0.8	0.8	0.4/0.8
P.rettgeri	0.4/0.8	0.4	0.2/0.4	0.4	0.8	0.8	0.8
P.aeruginosa	0.8/1.0	0.8	0.4/0.8	0.8	>1	>1	0.8/1
*C.albicans***	0/4	0.2/0.4	0.2	0.2/0.4	0.4/0.8	0.4/0.8	0.4/0.8

* When MIC equals MBC, only one value is presented, against;

** Antimicrobial activity against *C. albicans* is expressed as MIC and MFC

Decreasing of total content of carvacrol and thymol in *Lamiaceae* essential oils resulted in decreasing of its antimicrobial activity. Comparing to thyme oil and savory oil, pimento oil demonstrated higher antimicrobial activity, but its activity was lower than the activity of oregano oil. Comparing to essential oils pure compounds demonstrated lower antimicrobial activity. Based on these results it can be assumed that antimicrobial activity of investigated essential oils is mainly caused by major constituents, but effect of some minor compounds can not be excluded. The results obtained also proved the statement for probable synergistic effect among major and minor essential oil constituents (4, 7). Antimicrobial activity of eugenol is slightly higher than antimicrobial activity of thymol and carvacrol. Antimicrobial activity of pure compounds depends on its chemical structure (2-4, 7). Increasing the number of double bounds and hydroxyl groups increased antimicrobial activity of the volatile compounds (2). Based on the chemical structure (**Fig. 1**) eugenol must be more active than thymol and carvacrol, which is in agreement with our results (4,6,7,14). Thymol and carvacrol demonstrated equal antimicrobial activity, which means that the position of hydroxyl groups have no effect on the activity of tested compounds. According to Knobloch et al. (6) and Vasquez et al. (22) thymol and carvacrol are the most active respiration and protein inhibitors of bacterial and fungal growth. One possible mechanism of antimicrobial action of eugenol and its cytochrome P-450 mediated conversion into cytotoxic quinine methide is presented in **Fig. 2** (14, 21).

The results obtained expand the possibilities for application of thyme oil, oregano oil, savory oil and pimento oil not only as flavoring agents, but even as prospective natural antimicrobials in foods. They are a possible alternative for partial replacement of currently used synthetic

food preservatives, which is in agreement with recommendations of WHO. On the other hand application of essential oils from spices instead dry and ground spices will decrease the risk of additional contamination of meat products, because it is well known that spices are highly contaminated with microorganisms.

Additional experiments for studying antimicrobial activity of essential oils from spices in refrigeratory conditions are in progress.

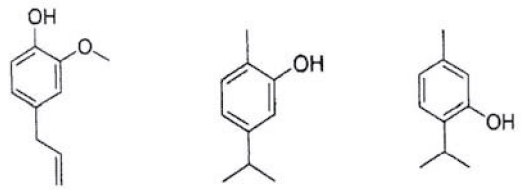

Fig. 1. Chemical structure of eugenol, carvacrol and thymol

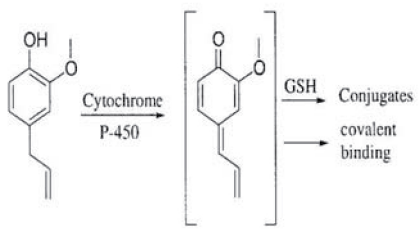

Fig. 2. Metabolic activation of eugenol and possible mechanism of action (14)

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