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ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS FROM SPICES AGAINST PSYCHROTROPHIC FOOD SPOILAGE MICROORGANISMS

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ABSTRACT

At current study the antimicrobial activity of Origanum vulgare, Satureja montana, Thymus vulgaris, Pimenta dioica and Syzygium aromaticum against psychrotrophic microorganisms, isolated from spoiled chilled meat products was investigated. MIC, MBC and MFC of the essential oils were determined both at 37° and 4°C. Antimicrobial activity of the essential oils retained unchanged at both temperatures. Among the tested psychrotrophic microorganism Gram-positive bacteria Brochothrix thermosphacta was the most sensitive strain and Gram-negative bacteria Pseudomonas aeruginosa was the most resistible. The results obtained expanded the possibilities for application of studied oils not only as flavour enhancers, but even as natural antimicrobials in chilled meat products.

Keywords: antimicrobial activity, essential oils, psychrotrophic bacteria

Introduction

Constantly increasing requirements of people to consume natural and safety foods without additional chemical flavour enhancers and synthetic preservatives, influenced scientists and industrial producers to search for natural compounds with wide spectrum of antimicrobial activities. Among the natural substances essential oils from spices are the most appropriate and promising natural antimicrobials, because they manifest their antimicrobial activity even at low concentrations, do not cause microbial resistance and undesired changes in foods, also are GRAS and commonly accepted by consumers (2,3). In addition to antimicrobial properties, essential oils demonstrated antioxidant, antiviral, antimycotic, antitoxigenic, antiparasitic and insecticidal action (1, 4, 13).

Psychrotrophic microorganisms associated with spoilage of chilled foods, especially meat and meat products, are one of the major problems of food industry. Microbial growth of spoilage and pathogenic psychrotrophic microorganisms and their metabolites caused undesired changes in organoleptic properties of foods and may be dangerous for human health (3).

Antimicrobial activities of essential oils from spices such as *Origanum vulgare*, *Thymus vilgaris*, *Satureja montana*, *Pimenta dioica* and other are widely studied, but usually they are tested *in vitro* at 30-35 °C, mainly against reference strains and by applying different antimicrobial testing procedures (4-6,8-14,18-22). On the other hand less or nothing is published about the antimicrobial action of essential oils from spices against food spoilage microorganisms at 4 °C. The mentioned reasons make many of the published results to a certain extent incomparable and impracticable in real foods and real industrial conditions.

The aim of present study is to investigate antimicrobial activity of essential oils from oregano, thyme, savory, pimento and clove against psychrotrophic spoilage microorganisms isolated from chilled meat products, both at 30°C and 4 °C.

Materials and Methods

Essential oil samples

The essential oils of oregano (O*riganum vulgare* L), savory (*Satureja montana*, L), thyme (*Thymus vulgaris*, L), pimento (*Pimenta dioica, L*) and clove (*Syzygium aromaticum* L), 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 (10).

Test microorganisms

Food isolates

The following test microorganisms, isolated from spoiled chilled meat products were used: *Pseudomonas fluorescens, P.putida, P.fragi, Brochothrix thermosphacta and Candida albicans*, The microbial isolates were identified to a species level on the basis of their physiological and biochemical properties in accordance with Bergey's Manual of Determinative Bacteriology. The strains are deposited in the microbial culture collection of Department "Biochemistry and microbiology" at The University of Plovdiv.

Reference strains

Antimicrobial activity of essential oils was also tested against the following reference strains: *P. fluorescens* ATCC 17397, *P. putida* NBIMCC 561, *P. aeruginosa* ATCC 9027, *C. albicans* ATCC 10231.

Preparation of test inoculums

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 28°C 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 standards. The yeast inoculum was prepared on the same way, but Sabouraud Agar (SA) and Sabouraud Broth (SA, SB, and NCPID) were used.

Antimicrobial testing procedures

Disc diffusion method

Disc diffusion method was carried out as described by Sacchetti et al. (21) in accordance with NCCLS recommendations (16). The Petri dishes (d= 90mm), containing solidified MHA or SA were inoculated by spreading 100μL of the inoculums. 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 divided into two groups. First group was incubated at 28°C for 24 h for bacterial cultures and 48 h for yeast cultures. Second group was incubated at 4°C for 24 h for bacterial cultures and 48 h for yeast cultures. 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 as described by Hili et al. (7) in accordance with NCCLS recommendations (15,17). A stock solution to be tested was prepared by diluting oil sample in DMSO (Sigma-Aldrich Co.). Stock solution was then added to culture broth to reach final oil concentrations ranging from 3.28% (w/v) to 0.01% (w/v). Serial dilutions were inoculated with 100μL of bacterial or yeast inoculums, prepared as listed above. The samples were divided into two groups. Firs group was incubated at 28°C for 24 h and 48 h for bacteria and yeasts, respectively, and the absorbance was read at 680 nm. Second group was incubated at 4°C for 24 h for bacterial cultures and 48 h for yeast cultures. 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 the 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 28°C for 24 h for bacterial cultures and 48 h for yeast

cultures. 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. Each experiment was performed in duplicate.

TABLE 1

Antimicrobial activity of essential oils, determined by disc diffusion method

Test microorganisms	IZ, mm				
	Oregano oil	Savory oil	Thyme oil	Clove oil	Pimento oil
Gram-negative					
P.fluorescens	22.8	20.4	22.3	10.0	10.0
P.fluorescens ATCC 17397	22.3	20.4	22.3	10.0	10.0
P.putida	21.3	15.3	22.0	10.0	11.0
P.putida NBIMCC 561	22.0	15.0	22.0	10.0	11.0
P.fragi	17.7	16.7	17.0	12.0	11.0
P.aeruginosa	8.0*	-	8.0	-	-
P.aeruginosa ATCC 9027	8.0	-	8.0	-	-
Gram-positive					
Broch. thermosphactaa	24.5	21.2	23.6	12.8	13.7
C.albicans	24.4	22.4	25.3	12.3	12.8
C.albicans ATCC 10231	22.7	20.5	22.8	12.2	12.0

* When IZ ≤ 8 mm, the oil was accepted as inactive

Results and Discussion

It is well known that antimicrobial activity of essential oils depends mainly on its chemical composition (23). Chemical composition of the studied essential oils was evaluated earlier and thymol was determined as the major constituent of thyme oil - 43.4%, carvacrol was the major constituent of savory oil and oregano oil - 41.5% and 66%, respectively and eugenol was the major constituent of pimento oil and clove oil - 76.02% and 76.84%, respectively (10). Agar disc diffusion method is recommended for preliminary testing of antimicrobial activity of natural compounds. 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 the studied essential oils demonstrated antimicrobial activity against all of the tested microorganisms, except both strains, belonging to species *P. aeruginosa*. Thyme oil and oregano oil demonstrated almost equal and the highest antimicrobial activity followed by savory oil, pimento oil and clove oil.

MIC, MBC and MFC of the essential oils were determined by serial broth dilution method at 28°C and 4°C. The results are shown in **Table 2** and **Table 3,** respectively.

As seen oregano oil and thyme oil demonstrated the highest antimicrobial activity followed by savory oil, clove oil and pimento oil. Antimicrobial activity of oregano oil slightly exceeded antimicrobial activity of thyme oil

probably, because of the higher total content of phenolic compounds such as carvacrol and thymol. Clove oil and pimento oil demonstrated almost equal antimicrobial activity, because they characterized with equal content of eugenol, which is the major constituent of both oils (23). Nevertheless that clove oil and pimento oil characterized with the highest content of phenolic compounds, in comparison with oregano oil, thyme oil and savory oil, they demonstrated weaker antimicrobial activity. Probably some of the minor compounds of these oils act antagonistic to the major compounds. Among the tested microorganisms Gram-positive *B. thermosphacta* was the most sensitive strain. Gram-negative bacteria belonging to genera *Pseudomonas* were more resistible and *P. aeruginosa* was the most resistible strain. The production of polysaccharide capsule by many strains belonging to *Pseudomonas spp*. probably is the main reason for the highest antimicrobial resistance of these strains, because the capsule impedes the diffusion of essential oil into the microbial cells. The studied essential oils also demonstrated antifungal activity against *C. albicans*. The results obtained are in accordance with published by Lambert et al (20) and our previous researches (10).

All of the studied essential oils demonstrated equal antimicrobial activity both at 4 °C and 37 °C, which means that the oils can be successfully applied in refrigerator conditions.

TABLE 2

MIC/MBC* and MIC/MFC of essential oils, determined by serial broth dilution method at 37°C

Test microorganisms	Oregano oil	Savory oil	Thyme oli	Clove oil	Pimento oil
Gram-negative					
P.fluorescens	0.05/0.1	0.2	0.05/0.1	0.2	0.2
P.fluorescens ATCC 17397	0.1	0.2	0.05	0.2	0.2
P.putida	0.1	0.1/0.2	0.05/0.1	0.2	0.2
P.putida NBIMCC 561	0.1	0.2	0.05/0.1	0.2	0.2
P.fragi	0.05	0.05	0.05	0.2	0.2/0.4
P.aeruginosa	0.4	0.4/0.8	0.4/0.8	≥0.8	≥0.8
P.aeruginosa ATCC 9027	0.4	0.8	0.8	≥0.8	≥0.8
Gram-positive					
Broch. thermosphacta	≤0.02	≤0.02	≤0.02	0.2	0.2
C.albicans	0.1/0.2	0.1/0.2	0.2	0.2	0.2/0.4
C.albicans ATCC 10231	0.2	0.2/0.4	0.2	0.2	0.2/0.4

*When MIC equals MBC only one value is published

Against *Candida* species antimicrobial activity is expressed as MIC and MFC

TABLE 3

MIC/MBC* and MIC/MFC of essential oils, determined by serial broth dilution method at 4° C

Test microorganisms	Oregano oil	Savory oil	Thyme oil	Clove oil	Pimento oil
Gram-negative					
P.fluorescens	0.05/0.1	0.1/0.2	0.05/0.1	0.2/0.4	0.2/0.4
P.fluorescens ATCC 17397	0.1	0.1/0.2	0.05/0.1	0.2/0.4	0.2/0.4
P.putida	0.1	0.1/0.2	0.05/0.1	0.2/0.4	0.2/0.4
P.putida NBIMCC 561	0.1	0.2/0.4	0.05/0.1	0.2/0.4	0.2/0.4
P.fragi	0.05	0.1/0.2	0.1	0.2/0.4	0.2/0.4
P.aeruginosa	0.4/0.8	0.4/0.8	0.8	0.4/0.8	≥0.8
P.aeruginosa ATCC 9027	0.4/0.8	0.8	0.8	≥0.8	≥0.8
Gram-positive					
Broch. thermosphacta	≤0.02	≤0.02	≤0.02	0.2	0.2
C.albicans	0.1/0.2	0.1/0.2	0.1/0.2	0.2	0.2/0.4
C.albicans ATCC 10231	0.2	0.2/0.4	0.1/0.2	0.2	0.2/0.4

* When MIC equals MBC only one value is published.

Against *Candida* species antimicrobial activity is expressed as MIC and MFC

Conclusions

The essential oils from oregano, thyme, savory, clove and pimento demonstrated high antimicrobial activity against all of the tested psychrotrophic microorganisms, isolated from meat products and reference strains. Antimicrobial activity of the essential oils retained unchanged both at 4 °C and 37 °C, which expand the possibilities for application of these oils not only as flavour enhancers, but even as natural antimicrobials in chilled products.

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