ACTA ABP BIOCHIMICA POLONICA

Vol. 62, No 4/2015
663–668
http://dx.doi.org/10.18388/abp.2015_1096

Regular paper

Hydrophobic properties of *Candida* spp. under the influence of selected essential oils*

Katarzyna Rajkowska✉, Alina Kunicka-Styczyńska and Marlena Pęczek

Institute of Fermentation Technology and Microbiology, Lodz University of Technology, Łódź, Poland

Processes of colonization of biotic and abiotic surfaces and biofilm formation depend inter alia on hydrophobic properties of *Candida* spp. The aim of this research was to determine the effect of tea tree, thyme and clove essential oils on hydrophobic properties of environmental and clinical *Candida* isolates. The relative cell surface hydrophobicity of strains tested was high, and ranged from 68.7% to 91.2%, with the highest value for a *C. rugosa* food-borne strain. The effectiveness of essential oils was diversified and depended on the type of essential oil, concentration and yeast strain. Statistically significant decrease of hydrophobicity indexes was observed after application of tea tree oil for *C. krusei*, clove oil for *C. albicans* reference strain, and all essential oils tested for *C. rugosa*. Only in the case of *C. famata* food-borne strain and *C. albicans* clinical isolate, solely used essential oils did not affect their hydrophobic properties. To determine the interactions of essential oils, their mixtures (1 MIC:1 MIC, 1 MIC:2 MIC and 2 MIC:1 MIC) were applied. Generally, essential oils used in combinations influenced yeast's hydrophobic properties much more than applied separately. The essential oils' mixtures reduced hydrophobicity of *Candida* yeasts in the range of 8.2 to 45.1%, depending on combination and strain. The interaction indexes of essential oils used in combinations predominantly indicate their additive effect. The application of tea tree, thyme and clove essential oils, especially in combinations, decreases hydrophobicity of the tested *Candida* isolates with implications of a probable advantageous limitation of their ability to colonize the food production industry environment.

Key words: *Candida* spp., food-borne and clinical strains, hydrophobic properties, essential oils

Received: 16 September, 2015; revised: 14 October, 2015; accepted: 03 November, 2015; vailable on-line: 24 November, 2015

INTRODUCTION

Candida spp. strains are characterized by their ability to form a biofilm structure on solid surfaces, which causes significant problems in many industrial branches, but is also threatening to human health (Kojic & Darouiche, 2004; Stratford, 2006). *Candida* biofilm is a heterogeneous, spatially well-organized structure consisting of planktonic and mycelial yeast forms which are interdependent in the quorum sensing system and surrounded by an extracellular polysaccharide substance (Chandra *et al*., 2001; Donlan, 2001). The process of biofilm formation can be divided into three stages: early, lasting up to 11 hours; intermediate, lasting between 12 and 30 hours; and maturation, lasting from 38 to 72 hours. During the first two hours, floating planktonic cells of *Candida albicans,* in blastospor forms, adhere to the materials' surfaces and the first microcolonies are noticeable after 3–4 hours after inoculation. The development of extracellular matrix with the cell wall polysaccharides containing mannose and glycosidic bonds, as the main component, dominates in the intermediate stage (Chandra *et al*., 2001). A further increase of extracellular matrix until complete surface covering by the colonies of *Candida* occurs during maturation of the biofilm structure stage.

Biofilm-forming microorganisms are characterized by high invasiveness, the ability to cause dangerous and difficult to treat infections (Donlan, 2001). Furthermore, the cells in the biofilm, compared to planktonic forms, showed reduced sensitivity to chemical compounds with antifungal activity and increased survival under unfavorable environmental conditions (Prażyńska & Gospodarek, 2014; Douglas, 2003). Currently available biofilm controlling methods rely on the prevention of surface contamination by following the principles of hygiene, the use of antifungal agents, the exchange of material, and minimizing yeast adhesion to the abiotic surface by the application of chemicals (Simoes *et al*., 2010). Limitation in the use of fungicides is caused by yeasts' resistance and potential risk to the humans exposed to the products of fungicide degradation (Krisch *et al*., 2011). Hence, a response to the increasingly sought after alternative natural compounds may be the essential oils. Essential oils (EOs) are plant-derived volatiles that can consist of more than 50 compounds, of which 1–3 are the main components representing 85–95% of the whole volume (Burt, 2004). The chemical profiles of the oils are crucial for their antimicrobial efficacy and the mechanism of action on the target organism. EOs are well known for their broad spectrum of antifungal activity, low toxicity, good biodegradability and safety (Kalemba & Kunicka, 2003).

The process of candidal adhesion, a prerequisite stage of the biofilm formation, is rather complex and involves both, biological and non-biological factors. The relative cell surface hydrophobicity of *Candida*, which is a property of the cell wall, is widely considered as a non-biological factor of critical importance pertaining to candidal adhesion (Ellepola & Samaranayake, 2001). In the light of potential use of essential oils as natural anticandidal agents, this study was undertaken to evaluate the effect

✉e-mail: katarzyna.rajkowska@p.lodz.pl

*The results were presented at the 6th International Weigl Conference on Microbiology, Gdańsk, Poland (8–10 July, 2015).

Abbreviations: EOs, essential oils; MIC, minimal inhibitory concentration; CSH, cell surface hydrophobicity; TTO, tea tree oil; HI, hydrophobicity index; FIH, fractional influence on hydrophobicity

of selected essential oils on hydrophobic properties of *Candida* strains of various origin.

MATERIALS AND METHODS

Yeast. The study was carried out for environmental and clinical *Candida* spp. isolates, namely *C. rugosa* fo/BG/05 (isolated from sauerkraut), *C. famata* fo/LI/02 (herring salad), *C. krusei* fo/MP/02 (sauerkraut) and *C. albicans* cl/MP/08 (faeces). Reference strain *C. albicans* ATCC 10231 was used for comparison. The strains were maintained on Sabouraud dextrose agar (peptone 10 g/l, dextrose 20 g/l, agar 20 g/l) and activated through double passaging in Sabouraud liquid medium at 37°C for 24 h.

Essential oils. The effect on *C. albicans* cells was estimated for essential oils of *Melaleuca alternifolia* (Maiden & Betche) Cheel (tea tree oil), *Thymus vulgaris* L. (thyme oil) and *Syzygium aromaticum* (L.) Merr. & L.M. Perry (clove oil), commercially produced and obtained from Pollena Aroma S.A. (Warsaw, Poland). Essential oils (EOs) were analyzed using Trace GC Ultra (Thermo Scientific) equipment combined with DSQ II mass spectrometer and with flame ionization detector (FID) and MS-FID splitter. Analysis was provided using a nonpolar chromatography column Rtx-1 ms (60 m × 0.25 mm, film thickness 0.25 μm, Restek). The oven temperature was programmed as follows: 50–300ºC at 4ºC/min; injector temp. 280°C; carrier gas helium with regular pressure 200 kPa, ionization energy 70 eV, ion source temperature 200°C. Identification of components was based on the comparison of their MS spectra with those of a laboratory made MS library, commercial libraries (NIST 98.1 and Mass Finder 4) along with the retention indices associated with a series of alkanes with linear interpolation (C8-C26). A quantitative analysis (expressed as percent ages of each component) was carried out by peak area normalization measurements without correction factors. The components of essential oils are presented in Table 1.

Table 1. Composition of examined essential oils (GC-MS analysis); RI – retention index, – not detected.

Compound	RI	Tea tree oil	Thyme oil	Clove oil
		Content (%)		
α-Thujene	926	0.8	0.9	–
α-Pinene	934	2.4	0.9	–
Camphene	940	–	0.4	–
Sabinene	968	0.1	–	–
β-Pinene	974	0.8	0.2	–
β-Myrcene	983	0.6	1.8	–
α-Phellandrene	996	0.5	0.3	–
Car-2-ene	1003	–	0.1	–
Car-3-ene	1008	–	2.0	–
α-Terpinene	1010	8.0	–	–
p-Cymene	1016	4.6	18.4	–
β-Phellandrene	1019	–	0.4	–
1,8-Cineole	1020	4.4	–	–
Limonene	1025	1.8	0.9	–
γ-Terpinene	1055	17.8	8.8	–
α-Terpinolene	1080	3.0	–	–
Linalool	1086	–	3.2	–
trans-p-Menth-2-en-1-ol	1112	0.3	–	–
cis-p-Ment-2-en-1-ol	1130	0.2	–	–
Borneol	1155	–	0.7	–
Terpinen-4-ol	1168	41.9	0.3	–
α-Terpineol	1178	3.8	0.3	–
cis-Piperitol	1202	0.1	–	–
Ascaridol	1207	0.3	–	–
Carvacrol methyl ether	1230	–	0.3	–
Cumin alcohol	1271	–	0.1	–
Thymol	1281	–	48.6	–
Carvacrol	1285	–	5.5	–
Eugenol	1342	–	–	85.2
α-Copaene	1374	0.2	–	–
Methyleugenol	1386	–	–	0.2
α-Gurjunene	1406	0.3	–	–
(E)-β-Caryophyllene	1421	0.3	2.3	9.9
Aromadendrene	1436	0.7	–	–
α-Humulene	1453	0.1	0.1	1.9
allo-Aromadendrene	1456	0.4	0.1	–
γ-Muurolene	1473	0.1	0.1	–
Ledene	1489	1.2	–	–
Viridiflorene	1490	–	0.1	–
α-Muurolene	1492	0.2	–	–
γ-Cadinene	1505	–	0.1	–
δ-Cadinene	1513	0.8	0.2	0.4
Spathulenol	1564	–	0.1	–
(E)-β-Caryophyllene oxide	1573	–	0.4	0.4
Globulol	1574	0.2	–	–

Cell surface hydrophobicity (CSH). The hydrophobicity of *Candida* strains was determined by microbial adhesion with a hydrocarbon method, according to Kanatiwela *et al.* (2013). The tested isolates were grown overnight in Sabouraud dextrose broth at 28°C and washed with phosphate buffered saline (NaCl 8 g/l, KCl 0.2 g/l, Na_2HPO_4 1.44 g/l, KH_2PO_4 0.24 g/l; pH 7.4). For adhesion assay, 2.5 ml of the cell suspensions (OD_{520} = 1.0) were mixed with 0.5 ml of p-xylene (Sigma-Aldrich) in an acid-washed glass tube. The samples were incubated at 37°C for 10 min and vigorously mixed for 30 sec. After 45 min of incubation at 37°C, the absorbance of aqueous phase was measured at 520 nm. The percentage of cells in the xylene layer was used to estimate the hydrophobicity index, HI, according to the formula:

$$HI = (A_1 - A_2 / A_1) \times 100\%,$$

where: A_1 – absorbance of inoculum, A_2 – absorbance of the aqueous phase.

The effect of essential oils on cell surface hydrophobicity of *Candida* spp. *Candida* strains' cell surface hydrophobicity was determined by incorporating essential oils in the inoculum, using the above procedure. As reference, antifungal antibiotics caspofungin acetate (1-[(4R,5S)-5-[(2-aminoethyl)amino]-N2-[(10R,12S)-10,12-dimethyl-1-oxotetradecyl]-4-hydroxy-L-ornithine]-5-(threo-3-hydroxy-L-ornithine pneumocandin B0 diacetate (Laboratories Merck Sharp & Dohme-Chibret, France) at the concentration of 5 μg/ml and nystatin (1S,3R,4R,7R,9R,11R,15S,16R,17R,18S,19E,21E,25E,27E,29E,31E,33R,35S,36R,37S)-33-[(3-amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,4,7,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo-nonatriaconta-19,21,25,27,29,31-hexaene-36-carboxylic acid (PPH Galfarm Sp z o.o., Poland) at the concentration of 50 μg/ml were used. EOs were applied at their MIC concentrations, specific for a particular strain and oil (Table 2). Each essential oil was used solely and in combinations (1 MIC:1 MIC, 2 MIC:1 MIC and 1 MIC:2 MIC).

In order to determine the interactions of essential oils in mixtures, the FIH (Fractional Influence on Hydrophobicity) were calculated, analogously to FIC (Fractional Inhibitory Concentration) (Silva *et al.*, 2011).

Table 2. Minimal inhibitory concentrations (MICs) of examined essential oils

Strain	Tea tree oil	Thyme oil	Clove oil
	Concentration (% v/v)		
fo/BG/05	0.125	0.125	0.125
fo/LI/02	0.25	0.03	0.03
fo/MP/02	0.25	0.03	0.06
cl/MP/08	0.125	0.25	0.125
ATCC 10231	2.0	0.25	0.25

FIH factors and FIH indexes (FIHi) were calculated, using the following formulas:

FIH of A oil = HI of mixture of oils/HI of A oil
FIH of B oil = HI of mixture of oils/HI of B oil
FIHi = FIH A + FIH B

Synergism was defined as FIHi ≤ 0.5; additivity as FIHi > 0.5 to < 2; indifference as FIHi ≥ 2 to < 4, and antagonism as FIHi ≥ 4 (Silva *et al*., 2011).

Statistical analysis of the results. Statistical calculations were carried out with the use of Statistica 6.0 software package (StatSoft). All results are expressed as the mean ± S.D. of 3 independent experiments. In order to compare the means, One-Way Anova test was performed at $p=0.05$ significance level using the statistical package of Origin version 6.1 (OriginLab Corporation).

RESULTS AND DISCUSSION

This study was performed for five *Candida* isolates, chosen conscientiously among several ones, each being a representative for their species (data unpublished). The specific *Candida* species were selected according to their different sensitivity to antibiotics and diverse biochemical profiles (Maroszyńska *et al*., 2013). In addition, among them there were both, food-borne and clinical isolates. Collating such a heterogenic biological material, we would also like to check the spectrum of the chosen EOs anticandidal activity in the light of their use against a variety of environmental strains. Four of the five strains tested showed, according to the accepted classification (Nostro et al., 2004), high hydrophobic properties and their hydrophobicity indexes ranged from 76.4 to 91.2%. On the other hand, *C. albicans* clinical isolate cl/MP/08 was characterized by a medium cell surface hydrophobicity (68.7 ± 6.0%). Previously, it has been demonstrated that hydrophobic yeasts are more virulent than their hydrophilic counterparts (Hazen & Hazen, 1992). However, even among clinical *Candida* strains their hydrophobic properties are diversified and may vary from 40% to 99% (Noumi *et al*., 2011). Much lower values of cell surface hydrophobicity, in the range from 2% to 41%, was obtained in a group of 50 *C. albicans* clinical isolates by Raut *et al.* (2010). Furthermore, hydrophobicity seems to be a species-dependent feature, and besides *C. albicans,* high values of hydrophobicity were displayed by *C. tropicalis*, both being the most clinically relevant *Candida* species (Noumi *et al*., 2011; Silva-Dias *et al*., 2015).

In the present study, changes in cell surface hydrophobicity of *Candida* strains were evaluated under the influence of essential oils with regard to antifungal antibiotics, nystatin and caspofungin. The choice of antibiotics resulted from the following reasons: nystatin is on the World Health Organization's List of Essential Medicines (2015) as one of the most efficacious, safe and cost-effective medicines needed in a basic health care system, and caspofungin is a valuable and fairly new generation antibiotic with a broad spectrum of anticandidal activity (Wieczorek *et al*., 2008). The effect of essential oils and antibiotics tested was diversified and depended on the type of antimycotics, their concentration and the yeast tested. This strain-dependent susceptibility to the essential oils' action was also previously observed for the clinical isolates within *C. albicans* species (Rajkowska *et al*., 2015). The range of hydrophobicity changes observed for *C. albicans* isolates (Rajkowska *et al*., 2015) were not statistically different from those observed here by us for the *Candida* species employed. The hydrophobic abilities of *Candida* yeasts seem to be rather strain- than species-dependent. For isolates of *C. rugosa* fo/BG/05, *C. famata* fo/LI/02, *C. albicans* cl/MP/08 and *C. albicans* ATCC 10231, statistically significant reduction in hydrophobic properties in the presence of essential oils was found (Fig. 1). Among these yeasts, the highest effect was expressed for *C. rugosa* fo/BG/05, and decrease in hydrophobicity index by 14.3 to 45.1% was observed under the influence of all the tested essential oils, used solely and in combinations, except for the mixture of tea tree and thyme oil (1 MIC: 2 MIC), as well as tea tree and clove oil (2 MIC: 1 MIC).

The statistically significant reduction, ranged from 17.6 to 38.2%, in HI values of *C. albicans* ATCC 10231 was noted for all essential oils' combinations, and additionally, for clove oil used solely (Fig. 1). Cell surface hydrophobicity of *C. albicans* cl/MP/08 clinical isolate was affected by six mixtures of essential oils, i.e. tea tree and thyme oil (1 MIC: 1 MIC, 1 MIC:2 MIC, 2 MIC: 1 MIC), tea tree and clove oil (1 MIC: 1 MIC), and thyme and clove oil (1 MIC:2 MIC, 2 MIC: 1 MIC), in a statistically significant way. In the case of *C. famata* fo/LI/02, only two combinations of tea tree and thyme oil (1 MIC: 1 MIC and 2 MIC: 1 MIC) caused statistically significant reduction in hydrophobicity of 21.6% and 24.0%, respectively. Only for *C. krusei* fo/MP/02, the effect of essential oils on the hydrophobicity was ambiguous, and in the presence of tea tree oil, and combination of thyme and clove oil (1 MIC: 2 MIC), a statistically significant decrease in hydrophobic properties of almost 9% was noted. Whereas, after treatment with thyme oil and mixtures of tea tree and thyme oil (1 MIC: 2 MIC), as well as tea tree and clove oil (1 MIC: 1 MIC), hydrophobicity increased by 3.1 to 7.2% (Fig. 1). Generally, essential oils used solely, influenced the yeast hydrophobic properties less than when applied in combinations.

Among the tested antibiotics, nystatin reduced hydrophobicity of four yeasts (*C. rugosa* fo/BG/05, *C. famata* fo/LI/02, *C. krusei* fo/MP/02 and *C. albicans* cl/MP/08) by 16.1 to 25.4%, depending on the strain (Fig. 1) in a statistically significant way. The application of caspofungin resulted in decrease in hydrophobic properties of three isolates (fo/BG/05, fo/MP/02, cl/MP/08) in the range of 14.7–6.3%. Interestingly, in our previous publication we have demonstrated that *C. famata* fo/LI/02 and *C. krusei* fo/MP/02 expressed an intermediate sensitivity to nystatin (Maroszyńska *et al*., 2013), and the current research indicates that even in those strains nystatin may reduce their hydrophobicity. However, the primary mechanisms of action of antibiotics tested are different and involve an increase of the cell membrane permeability by nystatin, and inhibition of the synthesis of β-(1,3)-D-glucan by caspofungin (Wieczorek *et al*., 2008).

Essential oils are complex mixtures of a wide diversity of components and their antimicrobial activity and mode

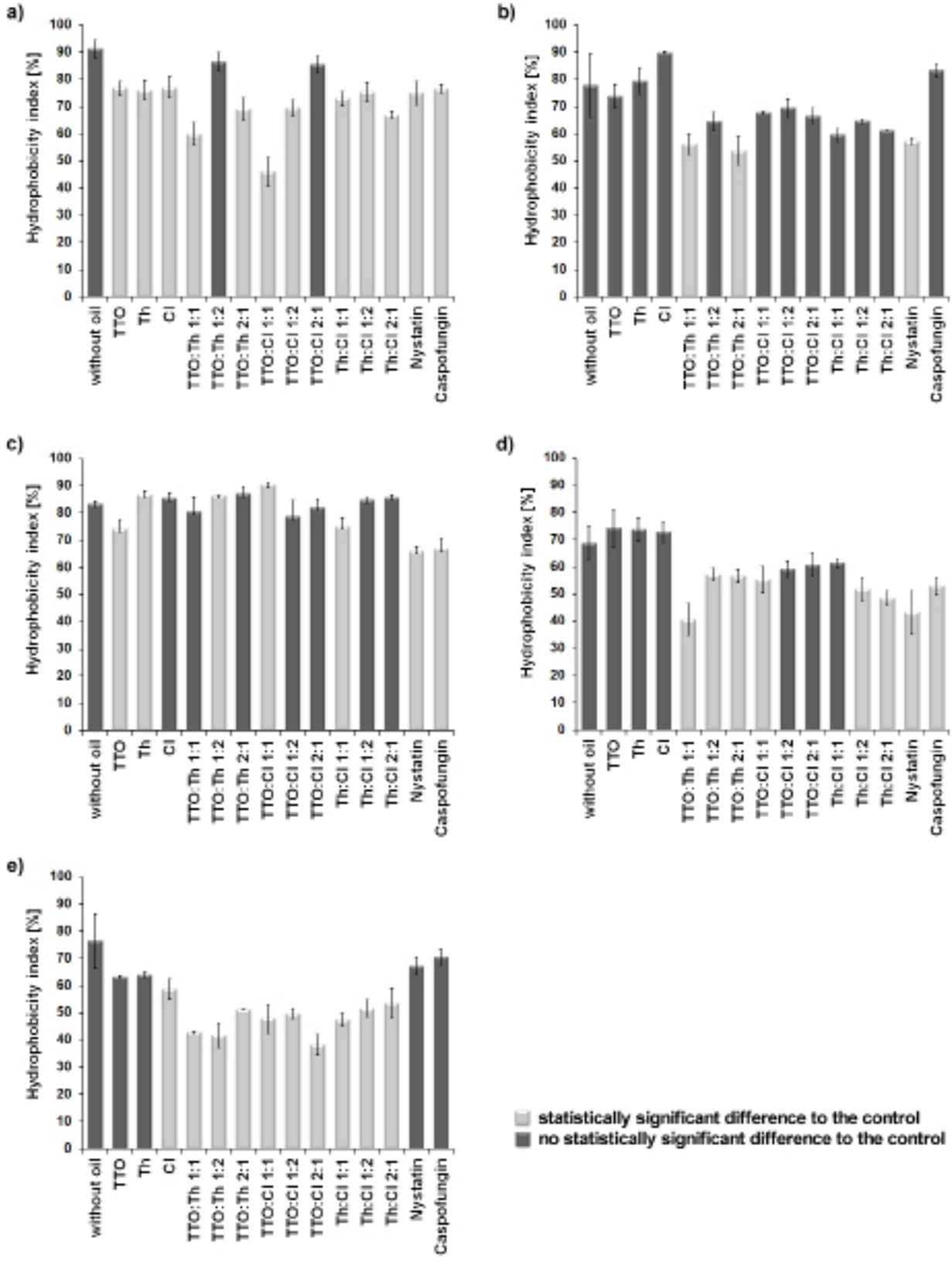

Figure 1. Changes in hydrophobic properties of *C. rugosa* fo/BG/05 (a), *C. famata* fo/LI/02 (b), *C. krusei* fo/MP/02 (c), *C. albicans* cl/MP/08 (d), *C. albicans* ATCC 10231 (e) under the influence of tea tree (TTO), thyme (Th) and clove (Cl) oils, used solely and in combinations (1 MIC:1 MIC, 1 MIC:2 MIC, 2 MIC:1 MIC), and antifungal antibiotics.

Table 3. Fractional influence on hydrophobicity indexes (FIHi) of examined essential oils' combinations; bold — additive effect.

EOs combinations	*C. rugosa* fo/BG/05	*C. famata* fo/LI/02	*C. krusei* fo/MP/02	*C. albicans* cl/MP/08	*C. albicans* ATCC 10231
	FIH index				
tea tree:thyme MIC:1 MIC	**1.6**	**1.5**	2.0	**1.1**	**1.3**
tea tree:thyme MIC:2 MIC	2.3	**1.7**	2.2	**1.5**	**1.3**
tea tree:thyme MIC:1 MIC	**1.8**	**1.4**	2.2	**1.5**	**1.6**
tea tree:clove MIC:1 MIC	**1.2**	**1.7**	2.3	**0.8**	**1.6**
tea tree:clove MIC:2 MIC	**1.8**	**1.7**	2.0	**1.6**	**1.6**
tea tree:clove MIC:1 MIC	2.2	**1.6**	2.1	**1.6**	**1.3**
thyme:clove 1 MIC:1 MIC	**1.9**	**1.4**	**1.7**	**1.7**	**1.6**
thyme:clove 1 MIC:2 MIC	2.0	**1.5**	2.0	**1.4**	**1.7**
thyme:clove 2 MIC:1 MIC	**1.7**	**1.5**	2.0	**1.3**	**1.7**

of action is therefore related to their composition, concentration and their possible interactions (Kalemba & Kunicka-Styczyńska 2003; Burt, 2004). In essential oil mixtures, four effects of their interaction can be highlighted: additive, antagonist, indifferent and synergistic. In our study, in the group of essential oil combinations, the additive effect was dominant (Table 3), which occurs when the combined effect of the components is equal to the sum of the individual effects (Lis-Balchin *et al*., 1998). Much less frequently, the use of oils in mixtures resulted in an indifferent effect, thus in these cases the activity of the combined substances was equal to the individual activities.

The literature data are focused on antimicrobial activity of essential oils and their components rather than on their influence on hydrophobic properties. It has been reported that EOs containing aldehydes or phenols, such as eugenol or thymol, as major compounds, showed the highest antimicrobial activity, followed by EOs containing terpene alcohols (Burt, 2004). Some studies have demonstrated that whole EOs usually have higher bioactivity than the mixtures of their major components, although interactions between components may lead not only to synergistic activity, but also to additive and antagonistic effects (Lambert *et al*., 2001; Burt, 2004; Bassolé & Juliani, 2012). Generally, compounds with similar structures exhibit additive rather than synergistic effects, and the occurrence of additive interactions of some EOs has been related to their major phenolic compounds (Lambert *et al*., 2001; Bassolé & Juliani, 2012). These findings may explain the predominant additive effect of essential oils tested in our study.

The relative cell surface hydrophobicity, CSH, of *Candida* spp. is considered to be a factor closely related to the adhesion properties of yeast (Ellepola & Samaranayake, 2001). The relationship between the hydrophobicity and enhanced adhesion of *Candida* species to plastic surfaces has been reported (Klotz *et al*., 1985). Some previous studies described a statistically significant positive correlation between CSH and candidal adhesion to buccal epithelial cells and denture acrylic surfaces (Samaranayake *et al*., 1995; Panagoda *et al*., 2000). It has been also reported, that biofilm biomass and biofilm formation has been associated with CSH (Blanco *et al*., 2010; Silva *et al*., 2011). On the other hand, *Candida albicans* is known to regulate cell surface hydrophobicity according to growth phase, environmental and nutritional conditions (Hazen *et al*., 1986).

Recently, a positive effect on *Candida* yeast cell surface hydrophobicity, adhesion abilities and biofilm formation has been demonstrated for variety of naturally derived substances or their constituents: eugenol (De Paula *et al*., 2014), human serum (Ding *et al*., 2014), *Lactobacillus*-derived biosurfactant (Ceresa *et al*., 2015), tyrosol (Monteiro *et al*., 2015), magnolol and honokiol (Sun *et al*., 2015), extracts of *Brucea javanica* and *Piper betle* (Nordin *et al*., 2013). Moreover, a positive correlation between the reduction in the CSH values and reduction of adhesion abilities and biofilm formation has been previously reported for *Candida* spp. (Borecká-Melkusová & Bujdáková, 2008; Nordin *et al*., 2013; De Paula *et al*., 2014). A significant decrease in the hydrophobicity (>40%), resulted in reduction in the number of adhered *Candida* cells to mammalian HEp-2 cells of up to 68.9% (De Paula *et al*., 2014), and to salivary pellicle of up to 86.0% (Nordin *et al*., 2013). Effectively reduced *Candida* CSH may also be related to limitation of biofilm formation by up to 66.7%. However, meaningfully reduced ability to form biofilm has also been noted for yeasts, which did not show changes in their hydrophobic properties (Borecká-Melkusová & Bujdáková, 2008).

An important issue is also the safety of essential oils and their components that are not orally administered. *In vivo* studies have demonstrated the safety of the topical use of eugenol and carvacrol, components of clove and thyme oils, for the treatment of vaginal (Chami *et al*., 2004) and oral (Chami *et al*., 2005) candidosis in rats. Toxicity of essential oils depends distinctly on concentration and the form of application (Hammer *et al*., 2006). It has been reported that the irritant capacity of tea tree oil, tested at concentration of 5% and 25% in cream, ointment and gel formulations, and in patients, who were patch tested with 10% TTO, was very low (Aspres & Freeman, 2003; Veien *et al*., 2004). Irritant and contact allergy reactions may be usually avoided through the use of lower concentrations of the irritant and therefore the use of 100% essential oils is not recommended (Hammer *et al*., 2006).

Due to the fact, that surface hydrophobicity of *Candida* spp. cells may affect cellular behavior and adhesion, the reduction of yeasts' hydrophobic properties may

limit their colonization abilities. In this context, the application of tea tree, thyme and clove essential oils, especially in combinations, may advantageously modulate the invasiveness of *Candida* yeasts by lowering their hydrophobicity. The mixtures of tea tree and thyme oils seem to be the promising agents with the spectrum of activity against all the *Candida* representatives tested. The presented results, demonstrating a decrease in surface hydrophobicity of food-borne strains, indicate a possibility of application of essential oils as antifungal agents under production conditions, notably in the food industry.

REFERENCES

Aspres N, Freeman S (2003) Predictive testing for irritancy and allergenicity of tea tree oil in normal human subjects. *Exogenous Dermatol* **2**: 258–261. http://dx.doi.org/10.1159/000078694.

Bassolé IHN, Juliani HR (2012) Essential oils in combination and their antimicrobial properties. *Molecules* **17**: 3989–4006. http://dx.doi.org/10.3390/molecules17043989.

Blanco MT, Sacristan B, Lucio L, Blanco J, Perez-Giraldo C, Gomez-Garcia AC (2010) Cell surface hydrophobicity as an indicator of other virulence factors in *Candida albicans*. *Rev Iberoam Micol* **27**: 195–199. http://dx.doi.org/10.1016/j.riam.2010.09.001.

Borecká-Melkusová S, Bujdáková H (2008) Variation of cell surface hydrophobicity and biofilm formation among genotypes of *Candida albicans* and *Candida dubliniensis* under antifungal treatment. *Can J Microbiol* **54**: 718–724. http://dx.doi.org/10.1139/w08-060.

Burt S (2004) Essential oils: their antibacterial properties and potential application in foods – a review. *Int J Food Microbiol* **94**: 223–253. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.022.

Ceresa C, Tessarolo F, Caola I, Nollo G, Cavallo M, Rinaldi M, Fracchia L (2015) Inhibition of *Candida albicans* adhesion on medical-grade silicone by a *Lactobacillus*-derived biosurfactant. *J Appl Microbiol* **118**: 1116–125. http://dx.doi.org/10.1111/jam.12760.

Chami F, Chami N, Bennis S, Trouillas J, Remmal A (2004) Evaluation of carvacrol and eugenol as prophylaxis and treatment of vaginal candidiasis in an immunosuppressed rat model. *J Antimicrob Chemother* **54**: 909–914. http://dx.doi.org/10.1093/jac/dkh436.

Chami N, Bennis S, Chami F, Aboussekhra A, Remmal A (2005) Study of anticandidal activity of carvacrol and eugenol *in vitro* and *in vivo*. *Oral Microbiology and Immunology* **20**: 106–111. http://dx.doi.org/10.1111/j.1399-302X.2004.00202.x.

Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormik T, Ghanoum MA (2001) Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture and drug resistance. *J Bacteriol* **183**: 5385–5394. http://dx.doi.org/10.1128/JB.183.18.5385-5394.2001.

De Paula SB,1 Bartelli TF, Di Raimo V, Santos JP, Morey AT, Bosini MA, Nakamura CV, Yamauchi LM, Yamada-Ogatta SF (2014) Effect of eugenol on cell surface hydrophobicity, adhesion, and biofilm of *Candida tropicalis* and *Candida dubliniensis* isolated from oral cavity of HIV-infected patients. *Evid Based Complement Alternat Med* **2014**: 8 pp, article ID 505204. http://dx.doi.org/10.1155/2014/505204.

Ding X, Liu Z, Su J, Yan D (2014) Human serum inhibits adhesion and biofilm formation in *Candida albicans*. *BMC Microbiol* **14**: 80–88. http://dx.doi.org/10.1186/1471-2180-14-80.

Donlan RM (2001) Biofilms and device-associated infections. *Emerg Infect Dis* **7**: 277–281.

Douglas LJ (2003) *Candida* biofilms and their role in infection. *Trends Microbiol* **11**: 30–36. http://dx.doi.org/10.1016/S0966-842X(02)00002-1.

Ellepola ANB, Samaranayake LP (2001) Investigative methods for studying the adhesion and cell surface hydrophobicity of *Candida* species: an overview. *Microb Ecol Health Dis* **13**: 46–54. http://dx.doi.org/10.3402/mehd.v13i1.7999.

Hammer KA, Carson CF, Riley TV, Nielsen JB (2006) A review of the toxicity of *Melaleuca alternifolia* (tea tree) oil. *Food Chem Toxicol* **44**: 616–625. http://dx.doi.org/10.1016/j.fct.2005.09.001.

Hazen KC, Hazen BW (1992) Hydrophobic surface protein masking by the opportunistic fungal pathogen *C. albicans*. *Infect Immun* **60**: 1499–508.

Hazen KC, Plotkin BJ, Klimas DM (1986) Influence of growth conditions on cell surface hydrophobicity of *Candida albicans* and *Candida glabrata*. *Infect Immun* **54**: 269–271.

Kalemba D, Kunicka-Styczyńska A (2003) Antibacterial and antifungal properties of essential oils. *Curr Med Chem* **10**: 813–829. http://dx.doi.org/10.2174/0929867033457719.

Kanatiwela DK, Abayasekara CL, Adikaram NKB, Parahitiyawa NB, Senanayake DM, Panagoda GJ (2013) Anti-candidal activity and effect on relative cell surface hydrophobicity of *Pongamia pinnata*. *Afr J Microbiol Res* **7**: 2948–2956. http://dx.doi.org/10.5897/AJMR2012.2386.

Klotz SA, Drutz DJ, Zajic JE (1985) Factors governing adherence of *Candida* species to plastic surfaces. *Infect Immun* **50**: 97–101.

Kojic EM, Darouiche RO (2004) *Candida* infections of medical devices. *Clin Microbiol Rev* **17**: 255–267. http://dx.doi.org/ 10.1128/CMR.17.2.255-267.2004.

Krisch J, Tserennadmid R, Vágvölgyi C (2011) Essential oils against yeasts and moulds causing food spoilage. In: *Science against microbial pathogens: communicating current research and technological advances*. Mendez-Vilas AM, eds, pp 1135–1142. Formatex, Spain.

Lambert RJW, Skandamis PN, Coote P, Nychas GJE (2001) A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J Appl Microbiol* **91**: 453–462. http://dx.doi.org/10.1046/j.1365-2672.2001.01428.x.

Lis-Balchin M, Deans SG, Eaglesham E (1998) Relationship between bioactivity and chemical composition of commercial essential oils. *Flavour Frag J* **13**: 98–104. http://dx.doi.org/10.1002/(SICI)1099-1026(199803/04)13:2<98::AID-FFJ705>3.0.CO;2-B.

Maroszyńska M, Kunicka-Styczyńska A, Rajkowska K, Maroszyńska I (2013) Antibiotics sensitivity of *Candida* clinical and food-borne isolates. *Acta Biochim Pol* **60**: 719–724.

Monteiro DR, Feresin LP, Arias LS, Barão VAR, Barbosa DB, Delbem ACB (2015) Effect of tyrosol on adhesion of *Candida albicans* and *Candida glabrata* to acrylic surfaces. *Med Mycol* **53**: 656–665. http://dx.doi.org/10.1093/mmy/myv052.

Nordin MA, Wan Harun WH, Abdul Razak F (2013) An *in vitro* study on the anti-adherence effect of *Brucea javanica* and *Piper betle* extracts towards oral *Candida*. *Arch Oral Biol* **58**: 1335–1342. http://dx.doi.org/10.1016/j.archoralbio.2013.07.001.

Nostro A, Cannatelli MA, Crisafi G, Musolino AD, Procopio F, Alonzo V (2004) Modifications of hydrophobicity, *in vitro* adherence and cellular aggregation of *Streptococcus mutans* by *Helichrysum italicum* extract. *Lett Appl Microbiol* **38**: 423–427. http://dx.doi.org/10.1111/j.1472-765X.2004.01509.x.

Noumi E, Snoussi M, Kammoun D, Bakhrouf A, Eulogio V (2011) Comparison of the adhesion ability of *Candida albicans* strains to biotic and abiotic surfaces. *Afr J Biotechnol* **10**: 977–985. http://dx.doi.org/10.5897/AJB10.992.

Panagoda GJ, Ellepola ANB, Samaranayake LP (2001) Adhesion of *Candida parapsilosis* to epithelial and acrylic surfaces correlates with cell surface hydrophobicity. *Mycoses* **44**: 29–35. http://dx.doi.org/10.1046/j.1439-0507.2001.00611.x.

Prażyńska M, Gospodarek E (2014) *In vitro* effect of amphotericin B on *Candida albicans*, *Candida glabrata* and *Candida parapsilosis* biofilm formation. *Mycopathologia* **177**: 19–27. http://dx.doi.org/10.1007/s11046-014-9727-7.

Rajkowska K, Kunicka-Styczyńska A, Pęczek M (2015) Hydrophobicity of clinical *Candida albicans* strains at the presence of thyme, clove and tea tree oils. 42nd Annual Conference on Yeasts, Smolenice, Slovakia Abstr. L4: 18.

Raut J, Rathod V, Karuppayil SM (2010) Cell surface hydrophobicity and adhesion: a study on fifty clinical isolates of *Candida albicans*. *Jpn J Med Mycol* **51**: 131–136. http://doi.org/10.3314/jjmm.51.131.

Samaranayake YH, Wu PC, Samaranayake LP, So M, Yuen KY (1995) Relationship between the cell surface hydrophobicity and adhesion of *Candida krusei* and *Candida albicans* to epithelial and denture acrylic surfaces. APMIS **103**: 707–713. http://dx.doi.org/10.1111/j.1699-0463.1995.tb01427.x.

Silva LV, Araújo MT, Santos KR, Nunes AP (2011) Evaluation of the synergistic potential of vancomycin combined with other antimicrobial agents against methicillin-resistant *Staphylococcus aureus* and coagulase-negative *Staphylococcus* spp strains. *Mem Inst Oswaldo Cruz* **106**: 44–50. http://dx.doi.org/10.1590/S0074-02762011000100007.

Silva-Dias A, Miranda IM, Branco J, Monteiro-Soares M, Pina-Vaz C, Rodrigues AG (2015) Adhesion, biofilm formation, cell surface hydrophobicity, and antifungal planktonic susceptibility: relationship among *Candida* spp. *Front Microbiol* **6**: 205. http://dx.doi.org/10.3389/fmicb.2015.00205.

Simoes M, Simoes LC, Vieira MJ (2010) A review of current and emergent biofilm control strategies. *LWT Food Sci Technol* **43**: 573–583. http://dx.doi.org/10.1016/j.lwt.2009.12.008.

Stratford M (2006) Food and beverage spoilage yeasts. In *Yeasts in food and beverages*. Querol A, Fleet GH, eds, pp 335–379. Springer-Verlag, Berlin, Heidelberg. http://dx.doi.org/ http://dx.doi.org/10.1007/978-3-540-28398-0.

Sun L, Liao K, Wang D (2015) Effects of magnolol and honokiol on adhesion, yeast-hyphal transition, and formation of biofilm by *Candida albicans*. PLoS ONE **10**: e0117695. http://dx.doi.org/10.1371/journal.pone.0117695.

Veien NK, Rosner K, Skovgaard G (2004) Is tea tree oil an important contact allergen? *Contact Dermat* **50**: 378–379. http://dx.doi.org/10.1111/j.0105-1873.2004.0350f.x.

WHO model list of essential medicines (2015) 19th Edition. World Health Organization.

Wieczorek P, Sacha P, Żórawski M, Jakoniuk P, Tryniszewska E (2008) *In vitro* activity of caspofungin against strains of *Candida*. *Mikol Lek* **15**: 135–139.