

Summary of PhD Thesis

Investigation of the disinfectant and antimicrobial action of essential oils suitable for food industrial application

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INTRODUCTION

Bacteria have the ability to attach to, and produce biofilm on, various surfaces occurring in food industry and health care. In that complex system bacteria are more resistant to harmful environmental effects. The first task in defence against biofilms is to inhibit adhesion. If the adhesion is irreversible the elimination is harder.

Bacteria can adapt to disinfectants used for longer times so that development of new agents may be necessary. Essential oils show antimicrobial, antifungal and antiviral effects and can hence be suitable to develop disinfection agents of novel type. Essential oils are derivated from plants. Most of the essential oils have the GRAS (generally recognized as safe) status by Food and Drug Administration (FDA).

AIMS

The general aim of our research work was to develop a disinfectant containing a natural active ingredient (essential oil) that is efficient in inhibition of development of bacterial biofilms on solid surfaces.

1. Investigation of the antimicrobial effect of the essential oils chosen.
2. Investigation and characterization of the antimicrobial effect of essential oil mixtures.
3. Investigation of the antiadhesive effect of essential oils on food industrial surfaces, including variations characteristic for the technological processes (surfaces in clean and food residue contaminated state).
4. Determination of the antibacterial kinetics of essential oils.
5. Investigation of the effect of the essential oil-based disinfectant liquid on biofilms of different maturation.
6. Removal of mixed population biofilms from tile, metal, plastic and wooden surfaces.
7. Optimization and proving of efficiency of the natural-based disinfectant.

METHODS

Investigated microorganisms: *Bacillus cereus* var. *mycoides* SZMC 0042, *Bacillus subtilis* SZMC 0209, *Escherichia coli* SZMC 0582, *Listeria monocytogenes* SZMC 21307, *Staphylococcus aureus* SZMC 110007, *Staphylococcus aureus* (MRSA) SZMC 6270, *Pseudomonas putida* SZMC 291T. Mixed culture: *E. coli*, *L. monocytogenes*, *P. putida*, *S. aureus*. Industrial isolates: *Citrobacter* sp., *Enterobacter* sp., *Escherichia* sp., *Pseudomonas* sp., *Serratia* sp.

Investigated essential oils: juniper, lemon, cinnamon, thyme, marjoram, clary sage.

Identification of the isolated bacteria:

- Sampling sites: Factory producing frozen pastries, training workshop producing meat products.
- Identification of the bacteria: purification of genomic DNA, agarose gel electrophoresis, PCR, sequencing and analysis of DNA fragments, analysis of nucleotide sequences (BLAST).

Determination of minimal inhibitory and bactericidal concentrations:

- Microdilution method.

Investigation of the interaction of essential oils

- Checkerboard method

Determining the adhesion ability of the bacteria

- On polypropylene and stainless steel surfaces, with hourly sampling over four hours,
- Essential oils used: marjoram, thyme, cinnamon,
- Food industrial model: how do food residues on the surfaces influence adhesion and the anti-adhesion effect of the essential oils,
- DAPI staining and observation under microscope.

Examination of the bactericidal kinetics of the essential oils:

- Varying of concentration and/or pH in order to shorten killing time.

Investigation and removal of single- and multiple-species biofilms:

- Formation of 24 and 168 hours biofilms on polypropylene, stainless steel, tile and wood surfaces,
- Disinfection of the surfaces with an essential oil containing solution according to the parameters (concentration, pH, action time) obtained in investigating the bactericidal kinetics,
- Determining surviving cell count on general and selective media.

Optimization of the disinfectant

- Application of the Box-Behnken model,
- Independent variables: concentration (1-3x MBC), time (10-110 min) and pH (4.5-7.5),
- Dependent variable: surviving cells in the biofilm,
- Bacteria: *E. coli* and *L. monocytogenes* on polypropylene surface; four-species mixed culture on polypropylene and wood surface,
- Comparison with some currently used disinfectants.

Checking the efficiency of the optimized disinfectant

- By removal of single- and multiple-species biofilms, 24 and 168 hours old.

Scanning electron microscopic imaging:

- 24 and 168 hours biofilms formed on plastic and wood surfaces,
- Scanning electron microscopic images from these biofilms.

RESULTS

The isolates belonged to the genera *Citrobacter*, *Enterobacter*, *Escherichia*, *Pseudomonas* and *Serratia*.

From the essential oils investigated, cinnamon-, marjoram- and thyme oil showed good antibacterial effect. The juniper-, lemon- and clary sage oils had limited effect; the minimum inhibitory concentration (MIC) was over 100 mg/mL for most of the bacteria. These EOs were omitted from further work. Generally, Gram-negative bacteria and industrial isolates were more resistant to the essential oils. Essential oils were very efficient also against MRSA and *Listeria monocytogenes*.

1. By examining the interactions of the essential oils it was found that the best results could be obtained if at least one of the oils had a phenolic component, such as thymol in thyme oil.

In most cases, additive or synergistic effect was achieved, except for the isolated strains in which case the essential oils had indifferent effect.

2. In sublethal concentrations, cinnamon, marjoram and thyme essential oils significantly reduced or inhibited the number of bacteria attached to stainless steel and polypropylene surfaces. The surface itself had no influence

on the attaching ability of the bacteria, but contamination with food residues greatly promoted attaching and decreased the effect of essential oils.

First of all, proteinous contaminations capable of covalently binding aldehydic components of the oils like cinnamaldehyde, caused decreased inhibitory effect. If proteins were removed from the contaminants, inhibitory effect was restored.

3. We developed cinnamon, marjoram and thyme essential oil based disinfectants which can satisfy the demand of industries.

4. Biofilm was eliminated from stainless steel and polypropylene surfaces with essential oil based disinfectant (mainly marjoram essential oil) at pH 4,5. Disinfection was more efficient on stainless steel than on plastic surface.

With aged biofilms, removal or cell count reduction was easier, possibly because of exhaustion of nutrients or accumulation of toxic substances. The best effect, that is, complete elimination of the biofilm, was achieved with marjoram oil at acidic pH but the other essential oils also showed their best effect in acidic solution. At low pH, most components of the essential oils are in non-dissociated state and strongly hydrophobic, enabling

them to cross the bacterial cell membrane and to disturb normal cellular functions.

5. The nature-friendly alternative antibacterial solution recommended by us is based on marjoram or cinnamon essential oil and has acidic pH (4.5), which are suitable for removal of single- or multi-species biofilms formed on stainless steel, polypropylene, tile, and wood surfaces. In industry and in households, single species biofilms are rare, mixed communities are much more typical. It was optimized applying the Box-Behnken experiment design. Regarding the hardest to disinfect wooden surfaces, **the recommended concentration of essential oil is 60 mg/mL for marjoram and 12.5 mg/mL for cinnamon, if the disinfectant is to be applied also in households. Disinfection time is 10 to 30 minutes depending on the surface.**

SUMMARY

- The investigated essential oils showed antibacterial effect, the most promising ones being cinnamon, thyme and marjoram.
- Synergistic or additive interaction was found with those essential oil mixtures where the main component of one member was a phenolic compound.
- There was no difference in the number of attached cells on polypropylene and stainless steel surfaces. Essential oils could diminish the number of attached cells, or even achieve complete inhibition of adhesion. Food residues on the surfaces promoted adhesion and reduced the effect of essential oils.
- Increasing the concentration and acidic or basic pH shortened the killing time of essential oils.
- Isolated bacteria were more sensitive than those from the culture collection.
- Disinfectants based on essential oils removed the biofilms from the surfaces.
- In four-species biofilms, the proportion of Gram-negative bacteria increased with time. Disinfection of wood surface was more difficult due to its porosity.
- The efficiency of the disinfectant obtained by optimization was equal to or better than that of household and industrial disinfectants.

I. THE PhD THESIS WAS BASED ON THE FOLLOWING PUBLICATIONS:

Anita Vidács, Erika Kerekes, Róbert Rajkó, Tamás Petkovits, Naiyf S Alharbi, Jamal M Khaled, Csaba Vágvölgyi, Judit Krisch. Optimization of essential oils-based natural disinfectants against *Listeria monocytogenes* and *Escherichia coli* biofilms formed on polypropylene surfaces. *Journal of Molecular Liquids* 255: pp. 257-262. (2018) ISSN 0167-7322 (IPF 3,648; Q1)

Erika-Beáta Kerekes, **Anita Vidács**, Julianna Jenei Török, Csilla Gömöri, Tamás Petkovits, Muthusamy Chandrasekaran, Shine Kadaikunnan, Naiyf S Alharbi, Csaba Vágvölgyi, Judit Krisch. Anti-listerial effect of selected essential oils and thymol. *Acta Biologica Hungarica* 67:(3) pp. 333-343. (2016). (IPF: 0,506; Q3)

II. FURTHER PUBLICATIONS RELATED TO THE PhD THESIS

a/ Publications in referred journals

Csilla Gömöri, **Anita Vidács**, Erika Beáta Kerekes, Elvira Nacsa-Farkas, Andrea Böszörményi, Csaba Vágvölgyi, Judit Krisch. Altered antimicrobial and anti-biofilm forming effect of thyme essential oil due to changes in composition. *Natural Product Communication* Vol. 13. In press (2018) (IPF:0,773)

b/ Other publications

Anita Vidács, Róbert Rajkó, Vágvölgyi, Judit Krisch. WASET (szerk.). Application of Box-Behnken Response Surface Design for Optimization of Essential Oil Based Disinfectant on Mixed

Species Biofilm. WASET, 2017. 1383 p. (International Science Index, Nutrition and Food Engineering, 11(12))

Vidács A, Rajkó R, Vágvölgyi Cs, Krisch J. Essential oil based disinfectants for biofilm elimination from stainless steel and plastic surfaces. In: 19th Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health: Program and abstracts. 65 p. (ISBN:978-963-306-535-8)

Anita Vidács, Judit Krisch, Csaba Vágvölgyi. Disinfection effect of essential oils on developing or matured bacterial biofilms. In: 30th EFFoST International Conference: Targeted Technologies for Sustainable Food Systems. Paper P2.68.

Anita Vidács. Essential oils as alternative disinfectants in the food industry. *Acta Biologica Szegediensis* 60:(1) p. 95. (2016). Conference For Doctoral Students In Biology.

Vidács A, Vágvölgyi Cs, Krisch J. Anti-adhesion effect of essential oils. In: International Conference on Science and Technique Based on Applied and Fundamental Research (ICoSTAF'16): Proceedings. Paper Vidacs_anti adhesion essential oils. 5 p.

Vidács A, Vágvölgyi Cs, Krisch J. Anti-adhesion effect of essential oils. In: Gábor Keszthelyi-Szabó, Cecília Hodúr, Judit Krisch (szerk.). International Conference on Science and Technique Based on Applied and Fundamental Research (ICoSTAF'16): Book of Abstracts. 58 p. (ISBN:978-963-306-482-5)

Anita Vidács, Judit Krisch, Muthusamy Chandrasekaran, Shine Kadaikunnan, Nayif S Alharbi, Csaba Vágvölgyi.

Potential use of essential oils for surface disinfection. In: 6th Congress of European Microbiologists (FEMS 2015). Paper FEMS-0987.

Anita Vidács, Judit Krisch, Csaba Vágvölgyi. Illóolajok felhasználása élelmiszeriparban használható fertőtlenítőszerekben. In: Gelencsér Éva, Horváth Zoltánné (szerk.). Aktualitások a táplálkozástudományi kutatásokban című V. PhD Konferencia összefoglalói. 36 p. (ISBN:978-963-88108-8-5)

Anita Vidács, Antal Véha, Ernő Gyimes, Csaba Vágvölgyi, Judit Krisch. New natural disinfectants: essential oils. In: 17th Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health: Program and Abstracts. 78 p. (ISBN:978-963-306-374-3)

Anita Vidács, Csaba Vágvölgyi, Erika Kerekes, Judit Krisch. Inhibition of bacterial attachment and biofilm formation on food industry surfaces using essential oils. *Natural Volatiles & Essential Oils* p.143.

Vidács A, Kerekes EB, Krisch J, Vágvölgyi C. Antibacterial effect of essential oil combinations. *Acta Microbiologica Et Immunologica Hungarica* 62:(S2) p. 239. (2015)

Anita Vidács, Antal Véha, Csaba Vágvölgyi, Judit Krisch. Antibacterial effect of selected essential oils as candidates for disinfectants in food industry. In: Second International Congress Food Technology, Quality and Safety. p. 18.

Vidács A, Krisch J, Vágvölgyi Cs. Disinfection action of some essential oils on stainless steel. In: Gábor Keszthelyi-Szabó,

Cecília Hodúr, Judit Krisch (szerk.). ICoSTAF'14: 56 p. (ISBN:978-963-306-276-0)

Vidács A, Krisch J, Vágvölgyi C. Disinfection action of some essential oils on stainless steel. *Review Of Faculty Of Engineering Analecta Technica Szegedinensia* 2014:(1) pp. 18-21. (2014). (ISBN 2064-7964)

Vidács A, Véha A, Vágvölgyi Cs, Krisch J. Antibacterial effect of selected essential oils as possible disinfectants in food industry. In: Second International Congress Food Technology, Quality and Safety. pp. 546-549.

Erika Beáta Kerekes, Csilla Gömöri, **Anita Vidács**, Elvira Nacsa-Farkas, Andrea Böszörményi, Nadeen Moustafa Zouabi, Csaba Vágvölgyi, Judit Krisch. Marjoram essential oil: changes in composition and effectiveness against bacterial biofilms. Fiala Biotechnológusok Országos Konferenciája, Abstract Book, p 95. ISBN 978-963-315-370-3 (2018)

Kerekes EB, **Vidács A**, Gömöri Cs, Nacsa-Farkas E, Takó M, Vágvölgyi Cs, Krisch J. Essential oils as food preservatives: from lab experiments to use in real foods. *Natural Volatiles & Essential Oils* 4:(3) p. 51. (2017).

Gömöri Cs, Nacsa-Farkas E, Kerekes EB, **Vidács A**, Bencsik O, Vágvölgyi Cs, Krisch J. Effect of cinnamon essential oil against aflatoxin production of *Aspergillus parasiticus*. In: ICoSTAF'16: Proceedings. Paper Gomori_cinnamon against aflatoxin production. 5 p.

Kerekes EB, Kósa V, **Vidács A**, Vágvölgyi Cs, Krisch J. Effect of selected essential oils and their component on

Debaryomyces hansenii biofilm formation. In: Gábor Keszthelyi-Szabó, Cecília Hodúr, Judit Krisch (szerk.). ICoSTAF'16: Book of Abstracts. 58 p. (ISBN:978-963-306-482-5)

Kerekes EB, Kósa V, **Vidács A**, Vágvölgyi Cs, Krisch J. Effect of selected essential oils and their componentst on Debaryomyces hansenii biofilm formation. In: ICoSTAF'16: Proceedings. Paper Kerekes_essential oils yeast biofilm. 5 p.

Kerekes EB, **Vidács A**, Gömöri Cs, Takó M, Vágvölgyi Cs, Krisch J. Essential oils as new alternatives for food preservation. In: Mrša V, Teparić R, Kifer D (szerk.). Power of Microbes in Industry and Environment 2016: Programme and abstracts. 130 p. (ISBN:978-953-7778-14-9)

Kerekes EB, **Vidács A**, Vágvölgyi C, Krisch J. Mixed culture biofilms: inhibition with essential oils and their main components. *Acta Microbiologica Et Immunologica Hungarica* 62:(S2) p. 163. (2015).

Kerekes EB, **Vidács A**, Török Jenei J, Gömöri C, Takó M, Chandrasekaran M, Kadaikunnan S, Alharbi NS, Krisch J, Vágvölgyi C. Essential oils against bacterial biofilm formation and quorum sensing of food-borne pathogens and spoilage microorganisms. In: Méndez-Vilas A (szerk.). The Battle Against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs. Badajoz: Formatex Research Center, 2015. pp. 429-437. (Microbiology Book Series; 5.) Volume 1. (ISBN:978-84-942134-6-5)

Török Jenei Julianna, Horváth Györgyi, **Vidács Anita**, Véha Antal, Krisch Judit. Antifungal activities of selected essential

oils. In: Second International Congress Food Technology, Quality and Safety. p. 191.

Török Jenei Julianna, Horváth Györgyi, **Vidács Anita**, Véha Antal, Krisch Judit. Antifungal activities of selected essential oils. In: Second International Congress Food Technology, Quality and Safety.pp. 550-553.

Krisch J, Kerekes E, Deák É, **Vidács A**, Vágvölgyi Cs. Antibiofilm forming and disinfectant effect of selected essential oils against mono- and mixed-culture bacterial biofilms. In: FEMS 2013 5th Congress of European Microbiologists. Paper 1077. 1 p.

III. FURTHER PUBLICATIONS

a/ Further publications in referred journals

Cs. Gömöri, E. Nacsá-Farkas, E. B. Kerekes, A. Vidács, O. Bencsik, S. Kocsubé, J. M. Khaled, N. S. Alharbi, Cs. Vágvölgyi, J. Krisch. Effect of essential oil vapours on aflatoxin production of *Aspergillus parasiticus*. *World Mycotoxin Journal* (2018) Accepted (**IPF: 2,189**)

b/ Conference abstracts

Gömöri Cs, Nacsá-Farkas E, Kerekes EB, **Vidács A**, Barna Zs, Róka E, Mészáros Basics B, Vágvölgyi Cs, Krisch. J. Water risk assesment - Legionella control in Hungary - results and experiences in our days. In: 19th Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health: Program and abstracts. 65 p. (ISBN:978-963-306-535-8)

Gömöri Cs, Nacsa-Farkas E, Kerekes EB, **Vidács A**, Barna Zs, Róka E, Mészáros Basics B, Póda T, Vágvölgyi Cs, Krisch J. Presence of Legionella in water samples from community and industrial facilities. In: 19th Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health: Program and abstracts. 65 p. (ISBN:978-963-306-535-8)

Kerekes EB, **Vidács A**, Takó M, Hargitai F, Komáromi L, Vágvölgyi Cs, Krisch J. Bacterial communication (quorum sensing) – impact on environment and health. In: 19th Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health: Program and abstracts. 65 p. (ISBN:978-963-306-535-8)

**Summed impact factor of the papers in referred journals:
7.116**