Research Article

Miroslava Kačániová*, Lucia Galovičová, Veronika Valková, Eva Tvrdá, Margarita Terentjeva, Jana Žiarovská, Simona Kunová, Tatsiana Savitskaya, Dmitrij Grinshpan, Jana Štefániková, Soňa Felsöciová, Nenad Vukovic, Przemysław Łukasz Kowalczewski*

Antimicrobial and antioxidant activities of *Cinnamomum cassia* essential oil and its application in food preservation

https://doi.org/10.1515/chem-2021-0191
received November 11, 2020; accepted January 4, 2021

Abstract: This study was designed to investigate chemical and antioxidant properties, as well as the antimicrobial and antibiofilm behaviour of *Cinnamomum cassia* essential oil (CCEO). MALDI-TOF MS Biotyper mass spectrometry was applied to evaluate the biofilms of *Stenotrophonomonas maltophilia* and *Bacillus subtilis*, while the antibiofilm ability of CCEO was assessed on wooden and glass surfaces. The antimicrobial activity by disc diffusion method, microdilution method, and vapour phase for two biofilm-producing bacteria and three *Penicillium* spp. were used. Antimicrobial and antibiofilm properties were assessed using the agar microdilution protocol. The vapour phase of *Penicillium citrinum*, *P. crustosum*, *P. expansum*, *S. maltophilia*, and *B. subtilis* on bread, carrot, potato, sweet potato, and apple *in situ* was studied. Specific molecular variations related to the biofilm formation and genetic analogies were evaluated with MSP spectra dendrograms of *S. maltophilia* and *B. subtilis* profiles were grown on different days. The results of disc diffusion and broth diffusion methods showed that CCEO was strongly effective against all tested microorganisms and the vapour phase method was effective and active against all *Penicillium* spp., but not strongly effective against bacteria in food preservation of food matrices.

*** Corresponding author: Miroslava Kačániová,** Department of Fruit Science, Viticulture and Enology, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976, Nitra, Slovakia; Department of Bioenergetics, Food Analysis and Microbiology, Institute of Food Technology and Nutrition, University of Rzeszow, Cwiklinskiej 1, 35-601, Rzeszow, Poland, e-mail: miroslava.kacaniova@gmail.com, tel: +421-641-4715

*** Corresponding author: Przemysław Łukasz Kowalczewski,** Department of Food Technology of Plant Origin, Poznań University of Life Sciences, 31 Wojska Polskiego St., 60-624, Poznań, Poland, e-mail: przemyslaw.kowalczewski@up.poznan.pl, tel: +48-61-848-7297

Lucia Galovičová: Department of Fruit Science, Viticulture and Enology, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976, Nitra, Slovakia, e-mail: l.galovicova95@gmail.com

Veronika Valková: Department of Fruit Science, Viticulture and Enology, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976, Nitra, Slovakia, e-mail: veronika.valkova@uniag.sk

Eva Tvrdá: Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976, Nitra, Slovakia, e-mail: eva.tvrda@uniag.sk

Margarita Terentjeva: Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, K. Helmaņa iela 8, LV-3004, Jelgava, Latvia, e-mail: margarita.terentjeva@llu.lv

Jana Žiarovská: Department of Plant Genetics and Breeding, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976, Nitra, Slovakia, e-mail: jana.ziarovska@uniag.sk

Simona Kunová: Department of Food Hygiene and Safety, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976, Nitra, Slovakia, e-mail: simona.kunova@uniag.sk

Tatsiana Savitskaya: Research Institute for Physical Chemical Problems, Belarusian State University, Leningradskaya str. 14, 220030, Minsk, Belarus, e-mail: savitskayaTA@bsu.by

Dmitrij Grinshpan: Research Institute for Physical Chemical Problems, Belarusian State University, Leningradskaya str. 14, 220030, Minsk, Belarus, e-mail: Grinshpan@bsu.by

Jana Štefániková: AgroBioTech Research Centre, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976, Nitra, Slovakia, e-mail: jana.stefanikova@uniag.sk

Soňa Felsöciová: Department of Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976, Nitra, Slovakia, e-mail: sona.felsociova@uniag.sk

Nenad Vukovic: Department of Chemistry, Faculty of Science, University of Kragujevac, P. O. Box 12, Kragujevac, Serbia, e-mail: nvchem@yahoo.com

Keywords: *Cinnamomum cassia*, antimicrobial activity, antibiofilm activity, bacteria, fungi, bread, vegetables, apple

1 Introduction

The genus *Cinnamomum* (Laureaceae family) consists of 250 species of wooden plants native to China, Southeast Asia, and Australia [1]. Trees and shrubs of the genus may be found in rainforests located within a wide range of altitudes, but are rare in latitudes with a typical seasonal climate [2]. Damp and well-drained locations are preferable for plant growth [2].

Essential oils (EOs) of *Cinnamomum cassia*, *Cinnamomum zeylanicum*, and *Cinnamomum camphora* are widely recognized for their various applications in the medicine and food industries [3,6]. The exact composition of the cinnamon EOs depends on the geographical origin and processing procedure. Cinnamon EOs have been used in medical remedies for centuries and their positive effects on the treatment of respiratory, gastrointestinal cardiovascular, and urinary disorders are well-described. EOs possess aphrodisiac, antihelminthic, antibacterial, insecticidal, antioxidant, antimutagenic activities, and tonic properties [3,6]. EOs from the leaves of *Cinnamomum osmophloeum* exhibit strong activity against bacteria, termites, mosquitoes, mildew, and other biological agents [4]. According to Verspohl et al. [5], the EO of *C. cassia* has expressed antidiabetic effects via the insulin-enhancing activity *in vitro* [6].

Cinnamon is a common ingredient in seasonings, sauces, bakery, confectionery, and drinks; the Food and Drug Administration has recognized cinnamon as a safe food additive [7].

Strong antifungal activity of cinnamon EOs was attributed to cinnamaldehyde abundance in the EO composition with up to 76.34% of the total of EO compounds. Antimicrobial activities against molds, e.g. *Rhizopus nigricans*, *Aspergillus flavus*, and *Penicillum expansum*, and bacteria, e.g. *Staphylococcus aureus* and foodborne pathogens, were studied previously [7–10].

Cinnamomum EOs may exhibit a significant antibiofilm activity: the EO from the trunk bark of *C. burmannii* interfered with planktonic cell growth and inhibited development of *S. aureus* and *P. aeruginosa* biofilms [11]. Trans-cinnamaldehyde has been shown to delay the formation of *E. coli* biofilm in urinary catheters [12].

The main objectives of this study were to study the chemical characteristics and antioxidant properties of *Cinamomum cassia* essential oil, the antibiofilm and molecular profile of biofilm formation of *C. cassia*, and the antimicrobial effectivity of *C. cassia* essential oil *in vitro* as well as *in situ*.

2 Materials and methods

2.1 Essential oil

Cinnamomun casia essential oil (CCEO) was obtained from Hanus, a.s. (Nitra, Slovakia). In our study, chemical characterization was performed and antioxidant activity of the essential oil was measured as well. Thereafter, the antimicrobial, antibiofilm activity, and molecular profile of biofilm were evaluated.

2.2 Chemical composition of the essential oil

Gas chromatographic-mass spectrometric analysis (GC Agilent 7890B and MS Agilent 5977A, Agilent Technologies Inc., Santa Clara, CA, USA) of CCEO was done as reported previously [13,14]. Prior to the analysis, a CCEO sample was diluted in hexane (HPLC ≥97%, Sigma Aldrich GmbH, Darmstad, Germany) to a concentration of 10 µL/mL. One microlitre of the diluted sample was injected into the inlet (250°C) operated in split mode 1:10. The separation was achieved using a HP-5 ms capillary column (30 m × 0.25 mm × 0.25 µm film; Agilent Technologies). The oven temperature program was set to 50°C for the first 5 min, and subsequently increased to 240°C at the rate of 3°C/min, where it was kept constant for 2 min. Helium was used as a carrier gas at a constant flow (1.2 mL/min). The mass detector parameters were as follows: ionization energy of the filament – 70 eV, transfer line temperature – 250°C, MS source temperature – 230°C, quadrupole temperature – 150°C. The mass spectrometer was programmed under electron impact (EI) in a full scan mode at m/z 40–350 with a scanning rate of 2.4 scans/s. The identification of compounds was carried out by comparing mass spectra (over 80% match) with a commercial database NIST® 2017, and Wiley library, retention times of reference standards (D-limonene, β-myrcene, and γ-terpinene; Sigma-Aldrich GmbH) comparison of data on the occurrence in CCEO with the literature [15–21]. The relative content of the identified compounds was calculated by dividing the individual peak area by the total area of all peaks. Each sample was measured in triplicate. The results

were expressed as the means of three injections ± standard errors (SE).

2.3 Radical scavenging activity – DPPH method

The radical scavenging activity of CCEO was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [13–22].

2.4 Microorganisms

Stenotrophomonas maltophilia and *Bacillus subtilis* were obtained from a milk producer, while *Penicillium expansum*, *P. crustosum* and *P. citrinum* were isolated from grapes. MALDI-TOF MS Biotyper (Brucker, Daltonic, Germany), 16S rRNA, and ITS sequencing were used for the identification.

2.5 Antimicrobial activity

The disc diffusion method was applied for detection of antimicrobial activity. *S. maltophilia* and *B. subtilis* were cultured in the Mueller Hinton broth (MHB, Oxoid, Basingstoke, UK) at 37°C overnight, while *P. expansum*, *P. crustosum*, and *P. citrinum* were incubated in Sabouraud dextrose broth (SDB, Oxoid, Basingstoke, United Kingdom) at 25°C for 48 h. Mueller–Hinton agar (MHA) and Sabouraud agar were inoculated with microbial suspension of tested species of 0.5 McFarland turbidity (densitometer Erba Lachema s.r.o., Brno, Czech Republic). Discs were impregnated with CCEO (10 µL/disc) and inoculates agars were incubated at 4°C for 1–2 h, later at 37 and 25°C for 18–24 and 48 h for bacteria and *Penicillium* spp., respectively. The zone of growth inhibition was measured. Fluconazole and chloramphenicol (30 µg, Oxoid, Basingstoke, UK) were used for the positive controls.

2.6 Minimum inhibitory and fungicidal concentration (MIC/MFB)

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MBC/MFC) were detected according to the National Committee for Clinical Laboratory Standards [23] as described by Kačániová et al. [13,14]. Chloramphenicol and nystatin, and DMSO served as positive and negative controls, respectively. MIC was detected at 570 nm with a spectrophotometer (Promega Inc., Madison, USA).

2.7 Minimum biofilm inhibitory concentration (MBIC)

MBIC against *S. maltophilia* was performed in microtitration plate [24]. Bacterial suspension preparation, incubation with CCEO, staining with crystal violet and acetic acid, and the evaluation of results were described previously [13,14,25]. MBIC was defined as the concentration of CCEO with absorbance less or equal to the negative control. The test was performed in triplicate.

2.8 Bread making process

The baking formula included 250 g wheat flour T650, 150 mL water, 2.5 g sucrose, 5 g salt, and yeast. The dough was fermented in a fermentation cabinet (MIWE cube; Pekass s.r.o., Plzeň; Czech Republic) at 32°C, with relative humidity 85% for 40 min. The loaves were baked at 180°C for 17 min with 160 mL of water, then at 210°C for 10 min in a steamy oven (MIWE cube). Freshly baked bread was left to rest at room temperature for 2 h.

2.9 Water activity and moisture content

Water activity (a_w) was assessed in cooled breadcrumbs (Lab Master a_w Standard, Novasina; Switzerland) at 25 ± 0.3°C. The moisture content was detected by moisture analyser (Kern DBS 60-3, Kern & Sohn GmbH; Germany) at 120°C.

2.10 *In situ* antifungal analysis of bread

The bread was sliced (150 mm of thickness) and placed into 0.5 L sterile glass jars (Bormioli Rocco, Italy). A 5 µL of suspension of fungal spores (1×10^6 spores/mL) in sterile PBS with 0.5% Tween 80 with density of 1–1.2 McFarland was applied for bread inoculation. CCEO concentrations of 125, 250, and 500 µL/L (EO + ethyl acetate) were used for the impregnation of sterile paper discs (6 cm). The discs were inserted into the jar lid and jars

were tightly closed. The sample material was incubated at 25 ± 1°C for 14 days in the dark and microbial colonies with visible mycelial growth were selected for confirmation [26].

2.11 Vapour phase of antimicrobial assay with vegetables and apple

An amount of 5 µL of inoculum was applied on 5 mm of thickness carrot slices, which were placed onto PDA (potato dextrose agar, Oxoid, Basingstoke, UK) agar. The dilution of CCEO in ethyl acetate (1:1) to a final volume of 125, 250, and 500 µL was used for the impregnation of a 55 mm sterile filter paper disc. After evaporation of ethyl acetate, the discs were placed between the lid and agar and incubated at 37°C for 18–24 h and at 25°C for 72 h for bacteria and fungi, respectively [14].

2.12 Biofilm development and molecular characteristics on different surfaces with MALDI-TOF MS biotyper

Pre-inoculated *S. maltophilia* and *B. subtilis* cultures were grown on a glass slide and a wooden toothpick with the addition of 1% CCEO. Sampling was done at 3, 5, 7, 9, 12, and 14 days of incubation on a shaker (170 rpm, 37°C) with a sterile cotton swab. The control samples were obtained from the planktonic cell suspension. MALDI-TOF MicroFlex analysis (Bruker Daltonics, Germany) with a standard global spectrum (MSP) produced by the MALDI Biotyper 3.0 software (Bruker Daltonics) was processed to the dendrogram method using Euclidean distances [27]. The obtained spectra were processed with FlexAnalysis 3.0 (Bruker Daltonics).

2.13 Statistical analysis

All experiments were performed in triplicate. Microsoft™ Excel® was used for data analysis. MBIC50 and MBIC90 (concentration for 50 or 90% reduction of bacterial biofilm) were evaluated with logit analysis.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Chemical characterization of *Cinnamomum cassia* essential oil (CCEO)

Earlier reports have shown that CCEO obtained from different parts of plants, e.g. leaves and bark, differ in their composition [28,29]. Age of the trees, growing seasons

Table 1: Chemical composition of *Cinnamomum cassia* essential oil (CCEO)

Compound name	RT[a]	Relative content (% ± SE)[b]
Cinnamene	7.97	0.18 ± 0.03
3-Carene	9.86	0.16 ± 0.02
Camphene	10.53	0.10 ± 0.01
Benzaldehyde	11.07	1.75 ± 0.13
o-Cymene	14.28	0.07 ± 0.00
D-Limonene	14.48	0.06 ± 0.00
Benzyl alcohol	14.75	0.08 ± 0.00
2-Hydroxy-benzaldehyde	15.12	0.80 ± 0.08
Acetophenone	16.33	0.08 ± 0.00
β-Phenethyl alcohol	18.68	1.29 ± 0.12
2-Methyl-benzofuran	20.12	0.37 ± 0.01
Benzenepropanal	21.11	1.01 ± 0.07
Endo-borneol	21.24	0.18 ± 0.02
3-Phenylpropanol	24.39	0.09 ± 0.01
2-Methoxy-benzaldehyde	24.91	0.84 ± 0.05
Phenetyl acetate	25.68	0.24 ± 0.02
Cinnamaldehyde	26.79	61.6 ± 2.58
Trans-cinnamyl alcohol	27.86	0.30 ± 0.04
Copaene	30.93	0.97 ± 0.05
Caryophyllene	32.77	0.20 ± 0.01
o-Hydroxy-cinnamic acid	33.45	4.12 ± 0.16
Cinnamyl acetate	33.91	5.35 ± 0.27
(+)-Ledene	34.48	0.20 ± 0.01
γ-Cadinene	35.16	0.21 ± 0.01
α-Curcumen	35.43	0.13 ± 0.03
β-Guaiene	35.90	0.10 ± 0.00
α-Muurolene	36.12	0.13 ± 0.00
β-Bisabolene	36.47	0.15 ± 0.01
β-Copaene	36.67	0.13 ± 0.00
Cadina-1(10),4-diene	37.05	0.34 ± 0.06
Trans-4-methoxycinnamaldehyde	37.43	13.8 ± 0.54
Farnesol	38.64	0.31 ± 0.01
(−)-Spathulenol	39.15	0.22 ± 0.01
β-Costol	39.36	0.25 ± 0.01
4-Epi-cubedol	41.58	0.08 ± 0.00
Benzyl benzoate	45.94	0.07 ± 0.00

[a] RT, Retention time (min). [b] Values represent means of three replicate determinations.

(a)

(b)

Figure 1: *In situ* antifungal assessment of bread inoculated with *Penicillium crustosum, P. expansum, and P. citrinum* in vapour phase. (a) control samples of bread; (b) bread inoculated with *P. crustosum, P. expansum, and P. citrinum* with the CCEO in concentration of 125, 250, and 500 µL/L.

or months, and sampled material (bark or xylem) were reported to affect the chemical composition of the CCEO [30–33]. Individual constituents of CCEO may be also affected by growing seasons or months for branches and leaves [32], as well as a natural variety of the sampled parts of the branches [33]. In our study, the main volatile compounds of CCEO were cinnamaldehyde (61.57%), trans-4-methoxycinnamaldehyde (13.78%), cinnamyl acetate (5.35%), and *o*-hydroxy-cinnamic acid (4.12%) (Table 1).

The main volatile compounds of the EO of bark at different growing seasons and age were trans-cinnamaldehyde (33.95–76.4%), cinnamyl alcohol acetate (0.09–49.63%), 2′-methoxycinnamaldehyde (0.09–6.69%), and copaene (1.09–14.3%) [15]. Phenolic materials with functionalized loop structures revealed higher antifungal and antibacterial activities [34]. Antifungal activity of cinnamaldehyde was reported previously [35]. Cinnamaldehyde (74–88%) has been found to be a major compound of CCEO [16,17] with wide application opportunities in medicine [18,19], food production, and chemical industry [20,21].

3.2 Antioxidant potential of CCEO

The DPPH radical inhibition value for CCEO was identified to be 42.04 ± 0.42%. The antioxidant properties of different parts of plant have been investigated with notable antioxidant properties reported [3]. Significant DPPH scavenging activity of *C. cassia* oil (92.4%) was detected previously [35].

3.3 Antimicrobial properties of CCEO

Strong antibacterial activity of CCEO was found against *Stenotrophomonas maltophilia* 27.33 ± 0.58 mm and

Bacillus subtilis (20.33 ± 1.53 mm). A lesser degree of activity was reported against *P. citrinum* (13.53 ± 1.15 mm), *P. crustosum* (10.64 ± 0.58 mm), and *P. expansum* (10.33 ± 1.53 mm). Antibacterial activity of CCEO against bacteria, including food-borne pathogens such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Streptococcus oralis*, *S. anginosus*, *Escherichia coli*, and *B. subtilis*, was reported [36–38]. Strong bacterial inhibitory effects in the present study support the findings on the antibacterial activities of CCEO. Antimicrobial activities were reported in other *Cinnamomum* plants, and Chinese cinnamon EO was reported to inhibit the growth of molds in foods with potential practical applications [39].

3.4 Minimum inhibitory concentration of CCEO

CCEO showed the highest activity against the *S. maltophilia* (MIC = 0.05 µL/mL) and against *B. subtilis* (MIC = 0.10 µL/mL). The high activity against *P. citrinum* (MFC =

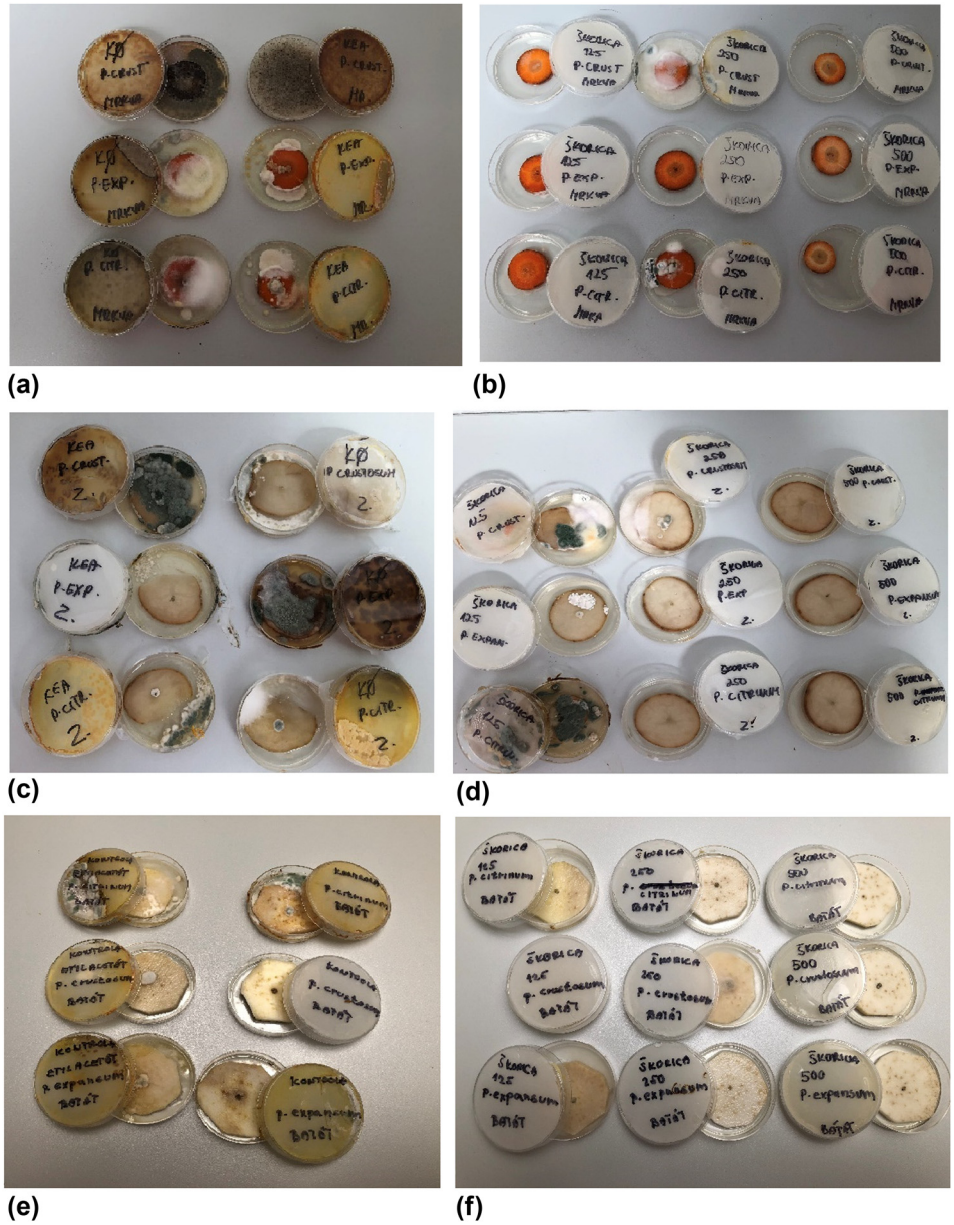

Figure 2: *In situ* antimicrobial evaluations of vegetables with *Penicillum* spp. in vapour phase. (a) control sample of carrot inoculated with fungi; (b) experimental group of carrot inoculated with *P. crustosum*, *P. citrinum*, and *P. expansum* at a concentration of 125, 250, and 500 µL/plate; (c) control sample of potato inoculated with molds; (d) experimental group of carrot with *P. crustosum*, *P. citrinum*, and *P. expansum* in a concentration of 125, 250, and 500 µL/plate; (e) control sample of sweet potato with inoculated with molds; (f) experimental group of sweet potato inoculated with *P. crustosum*, *P. citrinum*, and *P. expansum* in concentration of 125, 250, and 500 µL/plate.

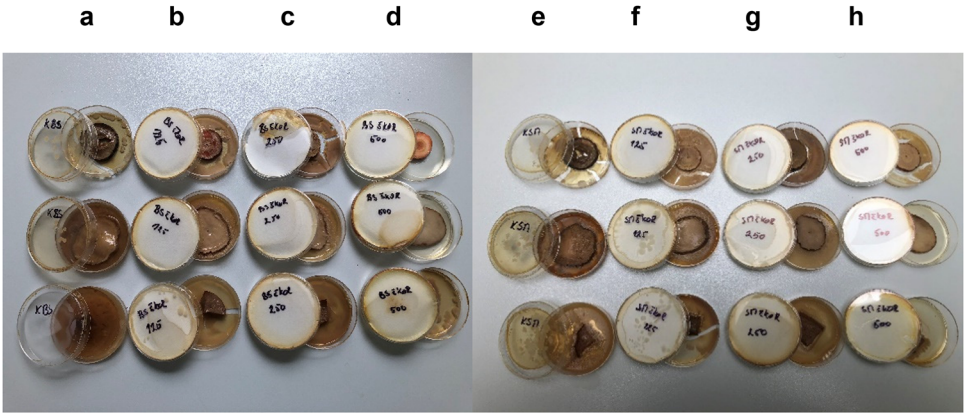

Figure 3: *In situ* antimicrobial activity of CCEO on vegetables in vapour phase. (a) control sample of carrot, potato, and apple contaminated with *B. subtilis*; (b) effect of CCEO at 125 µL/plate on carrot, potato, and apple contaminated with *B. subtilis*; (c) effect of CCEO at 125 µL/plate on carrot, potato, and apple contaminated with *B. subtilis*; (d) effect of CCEO at 125 µL/plate on carrot, potato, and apple contaminated with *B. subtilis*; (e) control sample of carrot, potato, and apple contaminated with *S. maltophilia*; (f) effect of CCEO at 125 µL/plate on carrot, potato, and apple contaminated with *S. maltophilia*; (g) effect of CCEO at 250 µL/plate on carrot, potato, and apple contaminated with *S. maltophilia*; (h) effect of CCEO at 500 µL/plate on carrot, potato, and apple contaminated with *S. maltophilia*.

0.78 µL/mL), *P. crustosum* (MFC = 0.39 µL/mL), and *P. expansum* (MFC = 0.20 µL/mL) was found which was in agreement with previous studies [36,38]. Our results showed that the best antimicrobial activity of CCEO was found against biofilm-producing strains of *S. maltophilia* and the worst antiicrobial activity was reported against microscopic filamentous fungus *P. citrinum*. The MIC extracts of *C. cassia* against *S. aureus* were in the range of 0.3–2.0 mg/mL [34]. Bud and bark extracts expressed higher antimicrobial activity against *S. aureus* and *A. baumannii* in comparison with the leaf extracts [34]. According to Manso et al. [40], the MIC and MFC of CCEO (100 and 200 ppm) were lower than 400 and 800 ppm recorded for oregano EO.

3.5 Moisture content and water activity

The moisture content of the bread was 42.23 ± 0.54% and water activity −0.9435 ± 0.005. Moisture content and water activity may alter the shelf-life of the bakery with elevated parameters and may enhance the microbial growth [41–44]. For white bread, the a_w value was reported to be within the range of 0.94–0.97 [45], making the product more susceptible to microbial spoilage, especially molds.

Intermediate moisture between 35 and 42% [46–49] is typical for bread, and that was in agreement with our data.

Aspergillus, *Rhizopus*, *Penicillium*, *Mucor*, *Monilia*, and *Eurotium* were common molds in bread [50]. *P. expansum* may withstand harsh environmental conditions which was a prerequisite of application of the mold in the experiment [51].

3.6 *In situ* antifungal activity of the CCEO in bread

MID50 and MID90 of the CCEO against *Penicillium citrinum* in the bread were 100.34 and 121.23 µL/L against *P. crustosum* of 121.12 and 135.25 µL/L, and against *P. expansum* of 101.12 and 119.84 µL/L, respectively. MID50 and MID90 for coriander EOs under identical conditions for same species of microscopic fungi were 367.19 and 445.92 µL/L [13]. MID50 and MID90 of the bitter orange EO against *P. crustosum* were 98.71 and 123.39, against *P. citrinum* of 136.52 and 188.40, and against *P. expansum* of 353.12 and 564.99, respectively [14] (Figure 1).

3.7 *In situ* antifungal activity of CCEO on vegetables

The highest antifungal activity of the CCEO on carrot was recorded against all tested fungi at a concentration of 125 µL/L, on potato at a concentration of 250 µL/L, and on sweet potato at a concentration of 500 µL/L (Figure 2a–f). The growth of *Aspergillus niger* was inhibited using 500, 1,000, and 2,000 µL of CCEO/L of air; doses of 300,

800, and 1,500 µL of CCEO/L of air were reported as the MIC against *P. expansum* [52].

3.8 *In situ* antibacterial activity of CCEO on carrot, potato, and apple

A higher antibacterial effect of CCEO at 500 µL/plate was recorded against *B. subtilis* (Figure 3a–h) on the carrot, potato, and apple. Contrasting results for *Citrus aurantium* essential oils (CAEO) were obtained in the previous *in situ* research; there the high antimicrobial potential of CAEO against *B. subtilis* was found to be at 62.5 µL/plate of CAEO concentration. EO of the bark of *C. cassia* regulated proliferation of *L. monocytogenes* in meats without sensorial changes of the product. Specifically, CCEO has reduced the microbial growth under laboratory conditions in comparison with naturally contaminated samples [53].

Previous studies were focused on the antibacterial activity of cinnamon against meat food-borne pathogens, including *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus*, *Arcobacter butzeiri*, and *Arcobacter skirrowii* [54–56]. In cheese, the antibacterial activity of the CEEO against *L. monocytogenes*, *S. aureus*, and *Salmonella enterica* was more profound at ~23°C, indicating a possible application as a natural food preservative [57].

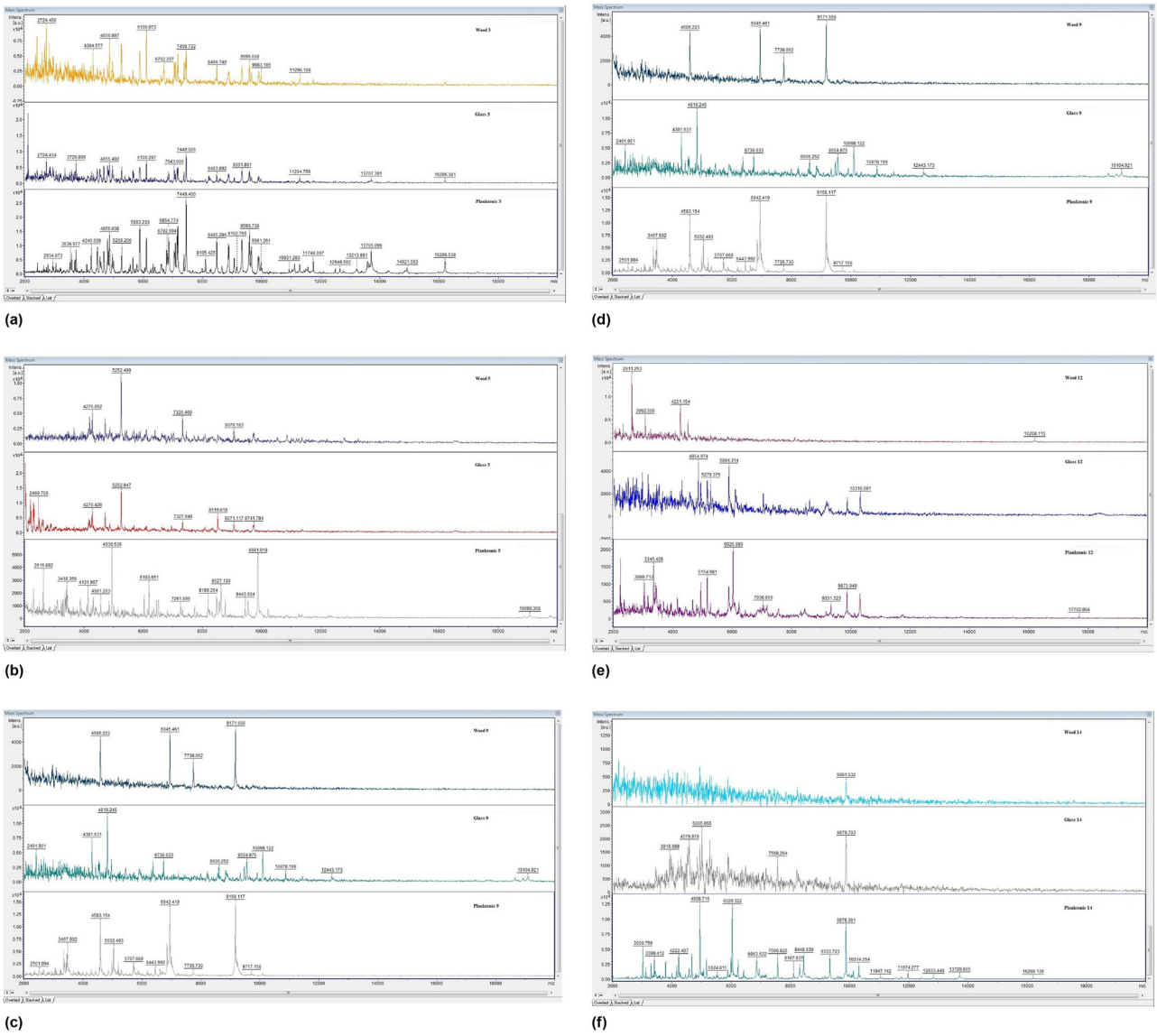

Figure 4: *B. subtilis* MALDI-TOF mass spectra after CCEO treatment, days of experiment: (a) 3th, (b) 5th, (c) 7th, (d) 9th, (e) 12th, (f) 14th.

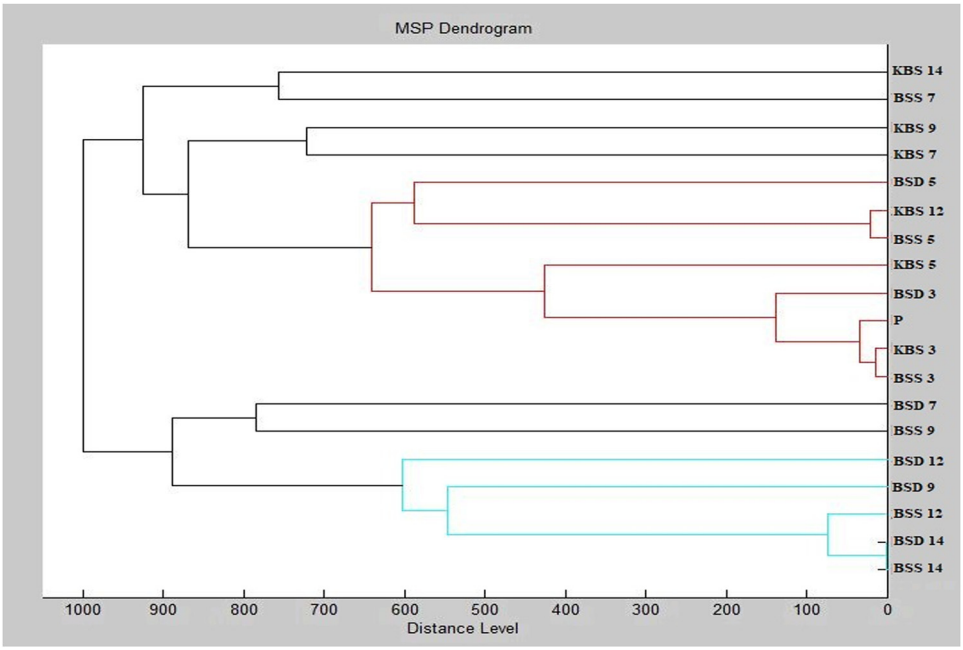

Figure 5: Dendrogram of *B. subtilis* generated using the MSP for the planktonic cells and all experimental groups. Sample name abbreviations: K, control; BS, *Bacillus subtilis*; S, glass; D, wood; P, planktonic cells.

The results of *in vitro* studies showed that the same concentrations of EOs exhibited different antifungal activity when tested on fruits *in vivo* [58]. Host/antifungal/pathogen interaction and different extrinsic factors can lead to divergent results in *in vitro* and *in vivo* experiments. Alteration of site action [59] or structural changes due to hydrolysis, degradation, and polymerization [60] of fruits under *in vivo* condition may explain the differences in recorded results. Similar results have been previously reported [61,62].

3.9 Antibiofilm properties of CCEO

MBIC50 and MBIC90 (minimal biofilm inhibition concentration) values were 3.71 and 5.36 µL/mL for *B. subtilis*, and 4.94 and 6.21 µL/mL for *S. maltophilia*, respectively. Commercially available *Cinnamomum zeylanicum* EO was effective in the inhibition of the biomass and viable counts of *P. aeruginosa* in biofilm at concentrations of 0.12–1.92 mg/mL. Biomass was completely inhibited at 1.92 mg/mL and the significant reduction of viable cells was recorded [63]. *C. zeylanicum* EO exhibited effect on 41.7 and 33.3% of *P. aeruginosa* and *S. aureus* biofilms [64].

3.10 Biofilm formation and molecular profile on surface following treatment with CCEO

The development of *B. subtilis* biofilm is shown in Figure 4. Spectra of the growing biofilms were presented in pairs for analysis of development on different surfaces.

Significant differences in spectra between the glass and planktonic spectrum were found on the 3rd day of experiment. Furthermore, significant differences between both experimental and control groups were identified on 5th day (Figure 4b). Differences between experimental and control groups were recorded during the 7–14th days of experiment (Figure 4c–f). The development stages of spectra of *B. subtilis* and *S. maltophilia* were similar to previously reported aftertreatment with different essential oils [13,14].

A dendrogram was constructed to make a grouping pattern of *B. subtilis* for analysed experimental groups (Figure 5). Two main clusters were generated at level 0.94 which were divided into six subclusters. No specific grouping was there, but most of glass samples together with control were grouped in the wider main cluster. The highest similarity of subclusters was seen for samples on the 3rd, 12th, and 14th day of experiment. All control groups were included in the same cluster, which showed

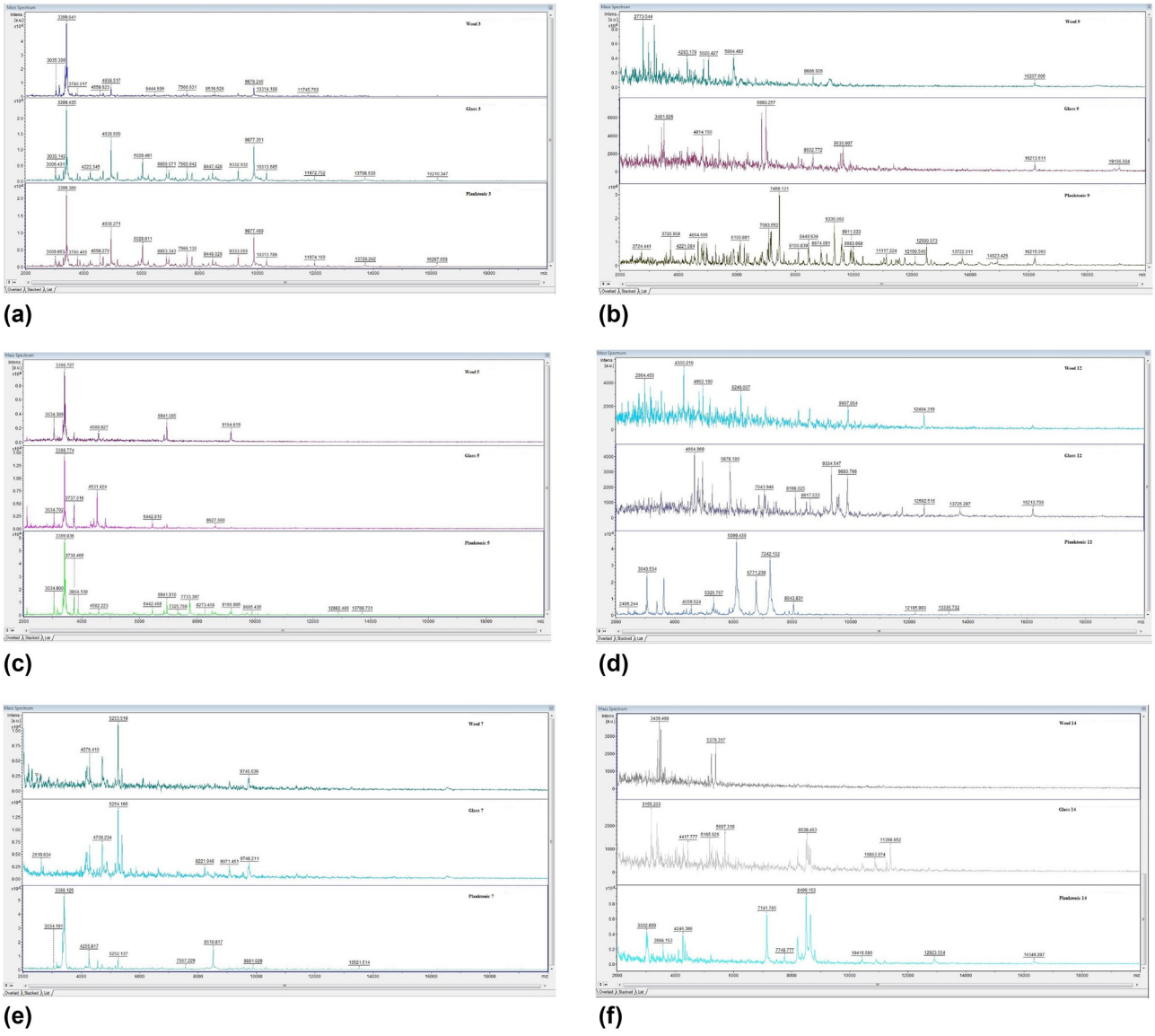

Figure 6: MALDI-TOF mass spectra of *S. maltophilia* after the CCEO treatment, days: (a) 3th, (b) 5th, (c) 7th, (d) 9th, (e) 12th, (f) 14th.

larger MSP distances when compared. The separation of the experimental group in the cluster was due to changes in the biofilm structure after the CCEO application.

MALDI-TOF mass spectra of *S. maltophilia* biofilms are shown in Figure 6. Spectra (a–f) were paired according to the day of biofilm development. Similarities between experimental and control samples were observed during the 3rd and 7th days of experiment (Figure 6a–c). Inhibition of pattern of experimental spectra was identified at the 9th up to the 12 days of the experiment (Figures 6d and e), after which CCEO interrupted the expansion of *S. maltophilia* biofilm. Spectra of the 14th day of experiment indicated complete degradation of the biofilm (Figure 6f).

The dendrogram of *S. maltophilia* consisted of three main clusters with the highest similarity of MSP found for control and wood samples on the 12th and 14th days of the experiment. The constructed dendrogram (Figure 7) shows the highest diversity for KSM samples on the 5th and 9th, and for SMD samples on the 3rd day of experiment. The other samples expressed relatively different spectra.

4 Conclusions

CCEO showed a satisfactory biological activity and strong inhibitory effect on microorganisms with significant influence in the food models. The main component of the CCEO was cinnamaldehyde which is known for strong

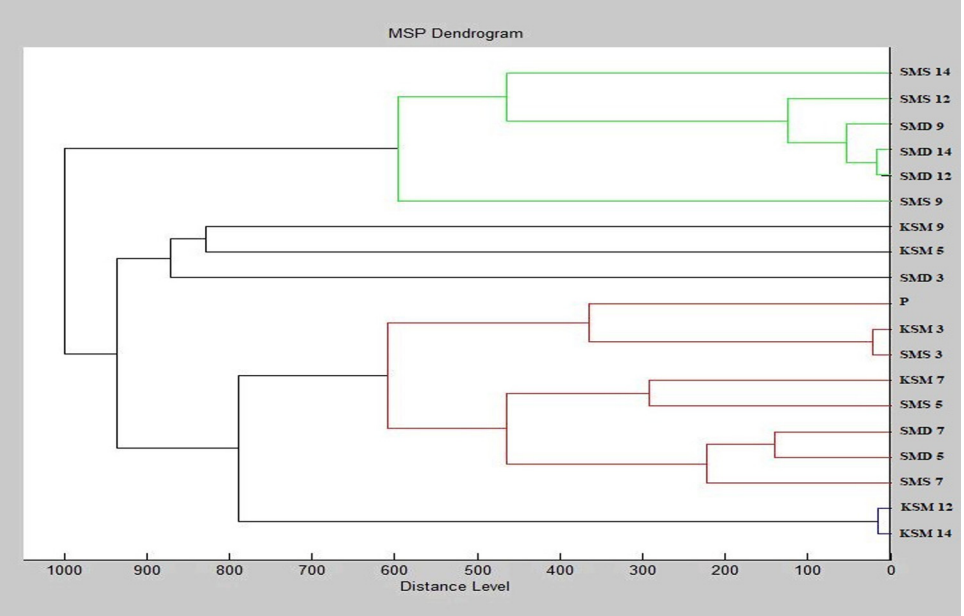

Figure 7: Dendrogram of *S. maltophilia* generated using MSP for the planktonic cells and all experimental groups. Abbreviations: K, control; SM, *Stenotrophomonas maltophilia*; S, glass; D, wood; P, planktonic cells.

inhibitory antimicrobial and antifungal action. The antibiofilm of CCEO was found to have the strongest antibiofilm action and was reported during the 7–14 days of experiments. Evaluation of CCEO revealed antioxidant and antimicrobial properties of CCEO. The vapour phase method is to be used for studies of inhibitory activities of bacteria and molds in the food model. According to the obtained results, CCEO could be suitable to reduce the damage caused by the fungi and biofilm-forming bacteria. EOs were obtained from edible plants and are safe for humans and environment. However, in order to be used as the organic alternative to chemical fungicides, deeper investigations about the absence of whatever form of toxicity are needed.

Acknowledgements: We would like to thank the grant of the VEGA no. 1/0180/20.

Funding source: This work was supported by the grant APVV SK-BY-RD-19-0014 and BRFBR No. X20SLKG-003 "The formulation of novel compositions and properties study of the polysaccharides based edible films and coatings with antimicrobial and antioxidant plant additives."

Author contributions: M. K., L. G., V. V., S. K., J. Š.: conceptualization; M. K., L. G., V. V., E. T.: formal analysis and methodology; M. K., L. G., V. V., S. K., J. Š.: data curation; M. K., L. G., V. V., E. T., M. T., J. Ž., S. K., T. S., D. G., J. Š., S. F., N. V., P. Ł. K.: writing original draft; M. K., M. T., E. T., P. Ł. K.: writing review & editing. All authors have carefully revised and approved the final version of the manuscript.

Conflict of interest: Przemysław Łukasz Kowalczewski, who is the co-author of this article, is a current Editorial Board member of Open Chemistry. This fact did not affect the peer-review process. The authors declare no other conflict of interest.

Data availability statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

[1] Leela NK. Cinnamon and cassia. In: Parthasarathy V, Chempakam B, Zachariah T, eds., Chemistry of spices. Boston, MA, USA: CABI; 2008. p. 124–45.

[2] Jantan I, bin Karim Moharam BA, Santhanam J, Jamal JA. Correlation between chemical composition and antifungal activity of the essential oils of eight Cinnamomum species. Pharm Biol. 2008;46:406–12. doi: 10.1080/13880200802055859.

[3] Kirtikar K, Basu B. Indian medical plants. 3rd edn., New Delhi, India: Goyal Offset Printers; 1984.

[4] Cheng S-S, Liu J-Y, Hsui Y-R, Chang S-T. Chemical polymorphism and antifungal activity of essential oils from leaves of different provenances of indigenous cinnamon (*Cinnamomum osmophloeum*). Bioresour Technol. 2006;97:306–12. doi: 10.1016/j.biortech.2005.02.030.

[5] Verspohl EJ, Bauer K, Neddermann E. Antidiabetic effect of *Cinnamomum cassia* and *Cinnamomum zeylanicum in vivo* and *in vitro*. Phyther Res. 2005;19:203–6. doi: 10.1002/ptr.1643.

[6] Unlu M, Ergene E, Unlu GV, Zeytinoglu HS, Vural N. Composition, antimicrobial activity and *in vitro* cytotoxicity of essential oil from *Cinnamomum zeylanicum* Blume (Lauraceae). Food Chem Toxicol. 2010;48:3274–80. doi: 10.1016/j.fct.2010.09.001.

[7] Xing Y, Li X, Xu Q, Yun J, Lu Y. Antifungal activities of cinnamon oil against *Rhizopus nigricans*, *Aspergillus flavus* and *Penicillium expansum in vitro* and *in vivo* fruit test. Int J Food Sci Technol. 2010;45:1837–42. doi: 10.1111/j.1365-2621.2010.02342.x.

[8] Reyes-Jurado F. Antifungal activity of essential oils from Mexican oregano and cinnamon in vapour phase. Mexico: Universidad de las Americas Puebla; 2013.

[9] Bouhdid S, Abrini J, Amensour M, Zhiri A, Espuny MJ, Manresa A. Functional and ultrastructural changes in *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells induced by *Cinnamomum verum* essential oil. J Appl Microbiol. 2010;109:1139–49. doi: 10.1111/j.1365-2672.2010.04740.x.

[10] Silveira SM, da Cunha Júnior A, Scheuermann GN, Secchi FL, Vieira CRW. Chemical composition and antimicrobial activity of essential oils from selected herbs cultivated in the South of Brazil against food spoilage and foodborne pathogens. Ciênc Rural. 2012;42:1300–6. doi: 10.1590/S0103-84782012000700026.

[11] Pratiwi SUT, Lagendijk EL, de Weert S, Idroes R, Hertiani T, Van den Hondel C. Effect of *Cinnamomum burmannii* Nees ex Bl. and *Massoia aromatica* Becc. essential oils on planktonic growth and biofilm formation of *Pseudomonas aeruginosa* and *Staphylococcus aureus in vitro*. Int J Appl Res Nat Prod. 2015;8:1–13.

[12] Kot B, Wicha J, Piechota M, Wolska K, Grużewska A. Antibiofilm activity of trans-cinnamaldehyde, p-coumaric, and ferulic acids on uropathogenic *Escherichia coli*. Turk J Med Sci. 2015;45:919–24. doi: 10.3906/sag-1406-112.

[13] Kačániová M, Galovičová L, Ivanišová E, Vukovic NL, Štefániková J, Valková V, et al. Antioxidant, antimicrobial and antibiofilm activity of coriander (*Coriandrum sativum* L.) essential oil for its application in foods. Foods. 2020;9:282. doi: 10.3390/foods9030282.

[14] Kačániová M, Terentjeva M, Galovičová L, Ivanišová E, Štefániková J, Valková V, et al. Biological activity and antibiofilm molecular profile of *Citrus aurantium* essential oil and its application in a food model. Molecules. 2020;25:3956. doi: 10.3390/molecules25173956.

[15] Geng S, Cui Z, Huang X, Chen Y, Xu D, Xiong P. Variations in essential oil yield and composition during *Cinnamomum cassia* bark growth. Ind Crop Prod. 2011;33:248–52. doi: 10.1016/j.indcrop.2010.10.018.

[16] Cheng B, Yu X, Ding J, Xu Y, Sun H, Ma X. China Cinnamomum plant resources and their aromatic constituents. Yunnan, China: Yunnan Science and Technology Press; 2001.

[17] Tan Y, Liu X, Yu ST. Study on the extraction of Cinnamon leaf oil from Cinnamon leaf by steam distillation. Food Res Dev. 2010;31:111–3.

[18] Vrinda Menon K, Garg SR. Inhibitory effect of clove oil on Listeria monocytogenes in meat and cheese. Food Microbiol. 2001;18:647–50. doi: 10.1006/fmic.2001.0430.

[19] Zhang K, Wei L, Shen H, Jiang L. Comparative study of inhibitive effect of cinnamaldehyde and citral upon Aspergillus niger growth. Chin J Microecol. 2011;23:141–3.

[20] Zhou J, Yang H, Li B, Cui Y. Food additives. Beijing, China: Chemical Industry Press; 2001.

[21] Ruan H. Application of cinnamaldehyde in perfume industry and daily chemical industry. Flavour Fragr Cosmet. 2005;2:37–8.

[22] Sánchez-Moreno C, Larrauri JA, Saura-Calixto F. A procedure to measure the antiradical efficiency of polyphenols. J Sci Food Agric. 1998;76:270–6. doi: 10.1002/(SICI)1097-0010(199802)76:2<270:AID-JSFA945>3.0.CO;2-9.

[23] NCCLS performance standards for antimicrobial susceptibility testing: eleventh informational supplement. Document M100-S11. PA, USA: National Committee for Clinical Laboratory Standard: Wayne; 2003.

[24] Ceri H, Olson ME, Morck DW, Storey DG. Minimal biofilm eradication concentration (MBEC) assay. Biofilms Infect Antimicrob Ther. 2005;257–69. doi: 10.1201/9781420028232.ch13.

[25] Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz J Infect Dis. 2011;15:305–11. doi: 10.1016/S1413-8670(11)70197-0.

[26] Oliveira SAC, Zambrana JRM, Di Iorio FBR, Pereira CA, Jorge AOC. The antimicrobial effects of *Citrus limonum* and *Citrus aurantium* essential oils on multi-species biofilms. Braz Oral Res. 2014;28:22–7. doi: 10.1590/S1806-83242013005000024.

[27] Pereira FDES, Bonatto CC, Lopes CAP, Pereira AL, Silva LP. Use of MALDI-TOF mass spectrometry to analyze the molecular profile of *Pseudomonas aeruginosa* biofilms grown on glass and plastic surfaces. Microb Pathog. 2015;86:32–7. doi: 10.1016/j.micpath.2015.07.005.

[28] Shen Q, Chen F, Luo J. Comparison studies on chemical constituents of essential oil from Ramulus Cinnamomi and Cortex Cinnamomi by GC-MS. Zhong Yao Cai. 2002;25:257–8.

[29] Wang R, Wang R, Yang B. Extraction of essential oils from five cinnamon leaves and identification of their volatile compound compositions. Innov Food Sci Emerg Technol. 2009;10:289–92. doi: 10.1016/j.ifset.2008.12.002.

[30] Xu Y, Cheng BQ, Ding JK, Yu Z, Chen ZH, Zeng JN. Investigation on Cinnamon resource, growth and yield of oil in Guangxi and Yunnan. Trop Agric Sci Technol. 2004;27:4–7.

[31] Huang YF, Huang JW, Tao L, Zhang YM. Chemical components of essential oils of *Cinnamomum cassia* Presl. in different growth year. Acta Sci Nat Univ Sunyatseni. 2005;44:82–5.

[32] Qin YR, Zhu JY, Zhang ZY, Zhang M, Qin MH. Annual variation laws of main chemical compositions and oil yielding rate in branches and leaves of *Cinnamomum cassia*. Nonwood Res. 2006;24:9–13.

[33] Lin J, Xu L, Liu J, Zou Z. Study on contents of cinnamaldehyde and cinnamic acid and distribution of *Ramulus Cinnamomi*. Chin Pharm J. 2005;40:1784.

[34] Yang C-H, Li R-X, Chuang L-Y. Antioxidant activity of various parts of *Cinnamomum cassia* extracted with different extraction methods. Molecules. 2012;17:7294–304. doi: 10.3390/molecules17067294.
[35] Feng T, Hu Z, Song S, Yao L, Sun M, Zhu X, et al. The antioxidant and tyrosinase inhibition properties of essential oil from the peel of Chinese *Torreya grandis* Fort. RSC Adv. 2019;9:42360–6. doi: 10.1039/C9RA06664K.
[36] Huang DF, Xu J-G, Liu J-X, Zhang H, Hu QP. Chemical constituents, antibacterial activity and mechanism of action of the essential oil from *Cinnamomum cassia* bark against four food-related bacteria. Microbiol. 2014;83:357–65. doi: 10.1134/S0026261714040067.
[37] Chaudhry NMA, Tariq P. Anti-microbial activity of *Cinnamomum cassia* against diverse microbial flora with its nutritional and medicinal impacts. Pak J Bot. 2006;38:169–74.
[38] Oussalah M, Caillet S, Saucier L, Lacroix M. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella* Typhimurium, *Staphylococcus aureus* and *Listeria monocytogenes*. Food Control. 2007;18:414–20. doi: 10.1016/j.foodcont.2005.11.009.
[39] Kocevski D, Du M, Kan J, Jing C, Lačanin I, Pavlović H. Antifungal effect of *Allium tuberosum, Cinnamomum cassia*, and *Pogostemon cablin* essential oils and their components against population of *Aspergillus* species. J Food Sci. 2013;78:M731–7. doi: 10.1111/1750-3841.12118.
[40] Manso S, Nerín C, Gómez-Lus R. Antifungal activity of the essential oil of cinnamon (*Cinnamomum zeylanicum*), oregano (*Origanum vulgare*) and lauramide argine ethyl ester (LAE) against the mold *Aspergillus flavus* CECT 2949. Ital J Food Sci. 2011;23:151–6.
[41] Lombard GE, Weinert IAG, Minnaar A, Taylor JRN. Preservation of South African steamed bread using hurdle technology. LWT Food Sci Technol. 2000;33:138–43. doi: 10.1006/fstl.1999.0626.
[42] Barbosa-Cnovas GV, Fontana AJ, Schmidt SJ, Labuza TP. Water activity in foods: fundamentals and applications. Oxford, UK: Blackwell Publishing Ltd; 2007.
[43] Cazier J-B, Gekas V. Water activity and its prediction: a review. Int J Food Prop. 2001;4:35–43. doi: 10.1081/JFP-100002187.
[44] Labuza TP, McNally L, Gallagher D, Hawkesh J, Hurtado F. Stability of intermediate moisture foods. 1. Lipid oxidation. J Food Sci. 1972;37:154–9. doi: 10.1111/j.1365-2621.1972.tb03408.x.
[45] Roos YH, Finley JW, DeMan JM. Water. In: DeMan JM, Finley J, Hurst WJ, Lee C, eds., Principles of food chemistry. Gaithersburg, MD, USA: Springer International Publishing; 1999. p. 606.
[46] Lahlali R, Serrhini MN, Jijakli MH. Studying and modelling the combined effect of temperature and water activity on the growth rate of *P. expansum*. Int J Food Microbiol. 2005;103:315–22. doi: 10.1016/j.ijfoodmicro.2005.02.002.
[47] Day L. Cereal food production with low salt. In encyclopedia of food grains. Amsterdam, The Netherlands: Elsevier; 2016. p. 396–402.
[48] Lee M-R, Swanson BG, Baik BK. Influence of amylose content on properties of wheat starch and breadmaking quality of starch and gluten blends. Cereal Chem J. 2001;78:701–6. doi: 10.1094/CCHEM.2001.78.6.7017016.
[49] Jaekel LZ, Silva CB, da Steel CJ, Chang YK. Influence of xylanase addition on the characteristics of loaf bread prepared with white flour or whole grain wheat flour. Food Sci Technol. 2012;32:844–9. doi: 10.1590/S0101-20612012005000116.
[50] Saranraj P, Geetha M. Microbial spoilage of bakery products and its control by preservatives. Int J Pharm Biol Arch. 2012;3:38–48.
[51] Quaglia M, Ederli L, Pasqualini S, Zazzerini A. Biological control agents and chemical inducers of resistance for postharvest control of *Penicillium expansum* link. on apple fruit. Postharvest Biol Technol. 2011;59:307–15. doi: 10.1016/j.postharvbio.2010.09.007.
[52] dos Santos NST, Aguiar AJAA, de Oliveira CEV, de Sales CV, e Silva SDM, da Silva RS, et al. Efficacy of the application of a coating composed of chitosan and *Origanum vulgare* L. essential oil to control *Rhizopus stolonifer* and *Aspergillus niger* in grapes (*Vitis labrusca* L.). Food Microbiol. 2012;32:345–53. doi: 10.1016/j.fm.2012.07.014.
[53] Dussault D, Vu KD, Lacroix M. *In vitro* evaluation of antimicrobial activities of various commercial essential oils, oleoresin and pure compounds against food pathogens and application in ham. Meat Sci. 2014;96:514–20. doi: 10.1016/j.meatsci.2013.08.015.
[54] Tayel AA, El-Tras WF, Moussa SH, El-Sabbagh SM. Surface decontamination and quality enhancement in meat steaks using plant extracts as natural biopreservatives. Foodborne Pathog Dis. 2012;9:755–61. doi: 10.1089/fpd.2012.1203.
[55] Chen CH, Ravishankar S, Marchello J, Friedman M. Antimicrobial activity of plant compounds against *Salmonella* Typhimurium DT104 in ground pork and the influence of heat and storage on the antimicrobial activity. J Food Prot. 2013;76:1264–9. doi: 10.4315/0362-028X.JFP-12-493.
[56] Irkin R, Abay S, Aydin F. Inhibitory effects of some plant essential oils against *Arcobacter butzleri* and potential for rosemary oil as a natural food preservative. J Med Food. 2011;14:291–6. doi: 10.1089/jmf.2010.0001.
[57] Shan B, Cai Y-Z, Brooks JD, Corke H. Potential application of spice and herb extracts as natural preservatives in cheese. J Med Food. 2011;14:284–90. doi: 10.1089/jmf.2010.0009.
[58] Tahmasebi M, Golmohammadi A, Nematollahzadeh A, Davari M, Chamani E. Control of nectarine fruits postharvest fungal rots caused by Botrytis cinerea and Rhizopus stolonifer via some essential oils. J Food Sci Technol. 2020;57:1647–55. doi: 10.1007/s13197-019-04197-4.
[59] Abdolahi A, Hassani A, Ghosta Y, Bernousi I, Meshkatalsadat M. Study on the potential use of essential oils for decay control and quality preservation of Tabarzeh table grape. J Plant Prot Res. 2010;50:45–52. doi: 10.2478/v10045-010-0008-2.
[60] Gatto MA, Ippolito A, Linsalata V, Cascarano NA, Nigro F, Vanadia S, et al. Activity of extracts from wild edible herbs against postharvest fungal diseases of fruit and vegetables. Postharvest Biol Technol. 2011;61:72–82. doi: 10.1016/j.postharvbio.2011.02.005.
[61] Mohammadi A, Hashemi M, Hosseini S. The control of Botrytis fruit rot in strawberry using combined treatments of Chitosan with *Zataria multiflora* or *Cinnamomum zeylanicum* essential oil. J Food Sci Technol. 2015;52:7441–8. doi: 10.1007/s13197-015-1871-7.

[62] Nikkhah M, Hashemi M, Habibi Najafi MB, Farhoosh R. Synergistic effects of some essential oils against fungal spoilage on pear fruit. Int J Food Microbiol. 2017;257:285–94. doi: 10.1016/j.ijfoodmicro.2017.06.021.
[63] Coelho FABL, Pereira MO. Exploring new treatment strategies for *Pseudomonas aeruginosa* bioflm infections based on plant essential oils. Microbial pathogens and strategies for combating tem: science, technology and education. Badajoz, Spain: Formatex; 2013.
[64] Sambyal SS, Sharma P, Shrivastava D. Anti-biofilm activity of selected plant essential oils against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Int J Curr Microbiol Appl Sci. 2017;6:444–50. doi: 10.20546/ijcmas.2017.603.051.