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Application of Three Types of Cinnamon Essential Oils as Natural Antifungal Preservatives in Wheat Bread

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Abstract: This research represents the report on the chemical profile, antioxidant, and antifungal (*Penicillium* (*P.*) *citrinum*, *P. expansum*, and *P. crustosum*) activities of three types of cinnamon essential oils (EOs), namely *Cinnamomum* (*C.*) *cassia* EO isolated from bark (CCEO), and two *C. verum* EOs isolated from plant bark (CVBEO) and leaf (CVLEO). The results revealed that the major compounds of the CCEO, CVBEO, and CVLEO were (*E*)-cinnamaldehyde (77.1%; 44.1%) and eugenol (70.8%), respectively; the demonstrable ($p < 0.05$) strongest antioxidant activity was detected in CVLEO (488.0 ± 1.2 TEAC; 84.0 ± 0.3%). The strongest *in vitro* antifungal activities were displayed by all analyzed EOs in the highest concentration (500 µL/L) used against *P. crustosum*, which inhibition zones ranged from 13.00 ± 1.73 mm (CVBEO) to 14.67 ± 1.15 mm (CCEO). Values for food model (bread) water activity and moisture content were 0.946 ± 0.002 and 40.88 ± 0.88%, respectively. *In situ* antifungal efficacies of all EOs examined were shown to be dose-dependent with the highest growth inhibition of mycelium determined in 250 µL/L of CVBEO against *P. citrinum* (95.23 ± 9.17%). The obtained findings promote the potential uses of the EOs and indicate their utilization for extending the shelf-life of bakery products.

Keywords: cinnamon essential oil; volatile compounds; antioxidant activity; disc diffusion method; microbial characterization; vapor contact method; anti-penicillium activity; bakery product

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1. Introduction

Bakery goods, especially bread, are the major products consumed around the world [1]. Wheat bread belongs to an intermediate-moisture food with moisture content (MC) typically varying from 35 to 42%; the water activity (a_w) of the product is above 0.95 [2]. Therefore, bread loaves are prone to mold spoilage (after a few days of storage) without the inclusion of control strategies, such as food preservatives or modified atmosphere packaging [3]. Concretely, *Penicillium*, *Cladosporium*, *Aspergillus*, *Neurospora*, and *Mucor* species have been noticed in bread loaves, being *Penicillium* identified as the most common source of their spoilage [4]. Moreover, fungal spoilage is responsible for the generation of unpleasant aroma and the formation of fungi secondary metabolites including mycotoxins, which can pose a risk to public health, and cause significant economic losses for both the consumers and the baking industry, as well [5]. Thus, it is necessary to search for suitable ways to extend the shelf-life of bakery goods. Essentially, consumer preferences associated with more natural and less processed foods encouraged the food industry to apply natural

antifungal substances [6]. This situation has forced the scientific community to research the innovative natural agents of herbs and aromatic plants including essential oils (EOs) [7].

Essential oils, also described as ethereal oils, are liquids containing aromatic and volatile substances naturally located in all plant parts, such as leaves, seeds, woods, barks, roots, flowers, and fruits [8]. Many techniques including solvent or supercritical fluid extractions, and hydrodistillation (HD) are used to isolate the oils from natural sources [9–11]. Although EOs consist particularly of terpenes, terpenoids, and phenylpropanoids, they represent a large variety of chemical structures, chemical compositions, and for that matter, an assortment of biological properties [12]. Particular attention is given to EOs as promising natural compounds with diverse properties including antifungal, antibacterial, antiviral, antioxidant, immune-modulatory, anti-inflammatory, anticancer, and analgesic actions [7,13]. In effect, it is known that the complex chemical structures of these natural substances are on a large scale responsible for their ability in modifying the microorganisms' membrane and cell wall, with consequent release of cell contents leading to their destruction [14]. Overall, 3000 EOs were identified to date from which about 300 types are being used in the commercial industry due to their beneficial characterization and pleasant aroma [15]. Cinnamon EO (CEO), one of the most popular EOs, is marked as safe by the Food and Drug Administration (FDA), and is greatly applied for active packaging in the food industry [16,17].

The plant genus *Cinnamomum*, frequently referred to as cinnamon (Lauraceae), consists of about 250 species including the economically important *Cinnamomum* (*C.*) *cassia* and *C. verum* (syn. *C. zeylanicum*) [18,19]. In the food industry (e.g., flavoring agents), the barks and leaves of the plants are mostly applied [20]. According to Hamidpour et al. [21], EOs obtained from these plants possess multiple pharmacological effects including antioxidant, antifungal, antibacterial, anti-diabetes, and anti-cholesterol properties. The effectiveness of CEO is conditioned by their chemical profile, which has been investigated in many research works [22,23]. Regarding the *C. verum* EO (CVEO), some studies have shown variability in its conception reflecting some chemical types [24]. The EOs obtained from bark (CVBEO) and leaf (CVLEO) of the plant growing in different world regions (Sri Lanka, India, Fiji Islands, and Malaysia) presented cinnamaldehyde and eugenol, respectively, as the major substances found in their chemical profile [25]. Similarly, in the case of EO isolated from *C. casia* (CCEO), Huang et al. [26] detected cinnamaldehyde as the main compound in its conception. In addition, frequent main substances of CEOs include camphor, cinnamyl-acetate, caryophyllene, trans α-bergamotene, caryophyllene oxide, linalool, geraniol, bornyl acetate, α-cubebene, γ-elemene, α-copaene, and guaiol, among others [27–29]. Regarding antimicrobial properties, EOs obtained from *C. cassia* and *C. verum* have been found to be effective in inhibiting the growth of various bacteria including Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Samonella typhymurium*) ones, and fungi including yeasts (four species of *Candida*, *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. krusei*), filamentous molds (*Aspergillus* spp. and *Fusarium* sp.) and dermatophytes (*Microsporum gypseum*, *Trichophyton rubrum* and *T. mentagraphytes*) [30]. Essential oil from *C. verum* has also been effective against multidrug-resistant strains of clinical Shigella isolates [31]. Furthermore, C. *cassia* EO displayed an inhibitory action against *Listeria monocytogenes* without creating strain resistance, as it was reported by Bermúdez-Capdevila [32], which is a critical finding for food preservation. In the study by Soliman and Badeaa [33], the EO (≤500 ppm) completely inhibited the growth of *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus*, and *Fusarium moniliforme* growing on potato dextrose agar medium. Moreover, CEO has been previously reported as a food preservative to inhibit fungal growth [34]. Indeed, to enhance the storage stability of food products, it has been applied in various fruits, such as apples [35], oranges [36], pomegranate [37], various types of meat [38,39], and dairy products [40,41]. Prior to our experiment, there are some papers revealing the effectivity of CEO application for bread preservation [42,43]; however, it is necessary to note that the antimicrobial activity of the same type of EO is

multifactorial-dependent. In effect, it is determined by the chemical composition of the EO which stronglydepends on many factors such as plant species and age, climate conditions, soil type, harvesting seasons, geographic region, and utilized extraction process [44]. All of the mentioned aspects may lead to different results between studies dealing with the same topic.

The principal objective of the current work was to assess the anti-penicillium activities of selected cinnamon EOs (CCEO, CVBEO, CVLEO) against three *Penicillium* strains using the vapor contact method. Moreover, the chemical profile of the analyzed EOs, their antioxidant, and *in vitro* antifungal activities, as well as basic technological properties and microbial characterization of wheat bread (as a food model for fungal growth in *in situ* conditions) were evaluated. In such a way, the EOs as natural preservative agents applied for the storage of bakery goods on a commercial scale can be assumed. In this regard, however, possible adverse effects of the EOs (especially in high concentrations) on food sensory (organoleptic) properties must also be taken into consideration. This investigation will be designed in our future experiments.

2. Materials and Methods

2.1. Analyzed EOs

Three types of cinnamon EOs (CEOs) obtained by steam distillation, namely *Cinnamomum* (*C.*) *cassia* EO isolated from bark (CCEO) and two *C. verum* EOs isolated from plant bark (CVBEO) and leaf (CVLEO) were employed for our analyses. The CEOs were purchased from the commercial producer Hanus Ltd. (Nitra, Slovakia), and until their next use, they were preserved in the laboratory refrigerator (at 4 °C). Essentially, the study completes our findings from our previous reports [45,46]. In this line, a comprehensive picture of the biological actions of different types of EOs purchased from the same commercial company can be drawn.

2.2. Assessment of EOs Chemical Profile

To evaluate the volatile substances of all EOs, GC-MS, i.e., gas chromatography (Agilent Technology 6890N, Agilent Technologies, Santa Clara, CA, USA) with mass spectrometry (quadrupole mass spectrometer 5975B, Agilent Technologies, Santa Clara, CA, USA) was employed. The experiment was performed according to the method described by Valková et al. [45].

2.3. Antioxidant Activity of EOs

The antioxidant activity (AA) of EOs was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay [30], and expressed as the percentage of DPPH inhibition, calculated according to the formula: $(A0 - A1)/A0 \times 100$; where A0 and A1 were the absorbance of DPPH and the sample, respectively.

The AA power was assessed in the following ascending manner: weak (0–29%) < medium-strong (30–59%) < strong (60 and more %). The values for total AA were also expressed as the Trolox equivalent antioxidant capacity (TEAC), i.e., in relation to the calibration curve as the standard reference Trolox (1 µg) to EOs samples (1 mL).

2.4. Antifungal Efficacies of EOs

2.4.1. Strains of Fungi

For determination of the antifungal potential of the analyzed EOs, three *Penicillium* strains (*P. expansum*, *P. crustosum*, *P. citrinum*) were applied. The microscopic filamentous fungi were isolated from *Vitis vinifera* berries (growing in Slovakian vineyards), classified using a reference-based MALDI-TOF MS Biotyper, and validated based on a comparison with the taxonomic identification using 16S rRNA (16S ribosomal ribonucleic acid) gene sequences analysis.

The fungal media were prepared according to the study by Valková et al. [45].

2.4.2. Antifungal (*In Vitro*) Properties of EOs

In vitro antifungal efficacy of analyzed EOs was evaluated by the agar disc diffusion technique, as previously described by Valková et al. [45]. In brief, an aliquot of culture media (100 µL) was inoculated on the SDA surface followed by the application of discs of filter paper (6 mm) impregnated with 10 µL of the EO samples (each in four concentrations: 62.5, 125, 250, and 500 µL/L, diluted in ethyl acetate). Consequently, the microscopic filamentous fungi were aerobically incubated at 25 ± 1 °C for a period of 5 days. After the process of incubation, the inhibition zone diameters (mm) were measured. The following ascending scheme was used to assess the inhibitory potential of the EOs: weak anti-penicillium efficacy (5–10 mm) < moderate anti-penicillium efficacy (10–15 mm) < very strong anti-penicillium efficacy (zone > 15 mm).

2.4.3. Antifungal (*In Situ*) Properties of EOs

To determine *in situ* antifungal activity of the EOs, *P. expansum*, *P. crustosum*, and *P. citrinum* were again used.

2.4.4. Food Model

Wheat bread loaves were selected as substrates for the *Penicillium* spp. growth. The bread samples were prepared in the Laboratory of Cereal Technologies (Research Center AgroBioTech, SUA in Nitra) as previously described by Valková et al. [47].

2.4.5. Food Model Moisture Content and Water Activity

The suitability of bread as a substrate for the growth of the fungi was predicted by measuring its MC (moisture analyzer DBS 60-3, Kern and Sohn, Ballingen, Germany) and water activity (a_w; Lab Master aw Standard analyzer, Novasina, Lachen, Switzerland).

2.4.6. Microbial Characterization of Bread Loaves during Their Storage

To determine microbial properties of bread samples during 14 days of storage, an aliquot amount of the bread samples (5 g) were homogenized with 45 mL of 0.89% physiological solution, and 100 µL of diluted sample was inoculated on Plate count agar (PCA, Oxoid, Basingstoke, UK), Violet Red Bile Salt Lactose agar (VRBL, Oxoid, Basingstoke, UK) and Potatoes dextrose agar (PDA, Oxoid, Basingstoke, UK), and aerobically cultivated for the total number of microorganisms (30 °C; 48–72 h), coliforms bacteria (37 °C; 24–48 h), and microscopic filamentous fungi (25 °C; 5 days), respectively. After the incubation process, colonies were separated from the plate, consequently re-inoculated on Tryptone Soya agar (TSA), and identified with MALDI-TOF MS Biotyper as was described [48]. All procedures were repeated on the first day and then for four weeks.

2.4.7. Vapor Contact Method

The method was carried out according to the methodology of Valková et al. [45]. First, the bread samples (15 mm of thickness) were inserted into glass jars (500 mL; Bormioli Rocco, Fidenza, Italy), and the inoculums of fungal strains tested were applied by stabbing with an injection pin on the three different places on the bread surface. Under the jar top, a sterile filter paper disc with a diameter of 60 mm was placed, and 100 µL of each EO (in four concentrations of 62.5, 125, 250, and 500 µL/L) was applied to the disc. The bread sample which was treated with no EO served as a control. All prepared jars were closed hermetically and then stored in an incubator (at 25 ± 1 °C) for two weeks.

2.4.8. Fungal Growth Inhibition

The growth of *Penicillium* strains on bread slices (*in situ* conditions) was stereologically estimated using ImageJ software (National Institutes of Health, Bethesda, MD, USA). In this regard, visible fungal colonies' volume density (Vv) was assessed using a point grid that counted the points falling to the colonies (P) and those (p) hitting the reference space (bread slice). This Vv was then calculated according to the formula: Vv (%) = P/p. Ultimately,

the antifungal potential of the EOs was expressed as the percentage of mycelial growth inhibition (MGI) as follows: FGI = [(C − T)/C] × 100, where C and T is the fungal growth (expressed as Vv) in the control and treated bread samples, respectively [30].

2.5. Statistical Analysis

The data was statistically evaluated by one-way analysis of variance (ANOVA) followed by Tukey's test using statistical software Prism 8.0.1 (GraphPad Software, San Diego, CA, USA). The level of significance was set to $p < 0.05$. All analyses were done in triplicate.

3. Results

3.1. Chemical Profile of EOs

The chemical composition of our CEOs was evaluated by GC-MS, and their individual chemical substances are listed in Table 1. The amounts of volatiles in percentage for each class of compounds are presented in Table 2. From the findings, it is evident that the identified substances represented 98.9%, 94.8%, and 99.1% of the oils from *C. cassia* bark, *C. verum* bark, and *C. verum* leaf, respectively. Non-terpenic compounds were abundant in the conception of the CCEO (94.8%) and CVBEO (51.0%), even though the CCLEO showed phenylpropanoids (72.9%) as the main constituents. Concretely, the major substances were shown to be (*E*)-cinnamaldehyde (77.1%), (*E*)-o-methoxy cinnamaldehyde (8.5%), and (*E*)-cinnamyl acetate (3.0%) in the CCEO; (*E*)-cinnamaldehyde (44.1%), eugenol (23.5%), and (*E*)-caryophyllene (3.9%) in the CVBEO; eugenol (70.8%), benzyl benzoate (3.9%), and (*E*)-caryophyllene (3.5%) in the CVLEO.

Table 1. Chemical profile of tested EOs.

No.	Compound [a]	EO1 [b]	Sample EO2 [c] % [g]	EO3 [d]	RI [e] (calc.)	RI [f] (lit.)
1	α-thujene	tr [h]	/ [j]	tr	926	930
2	α-pinene	0.6	0.8	1.8	938	939
3	camphene	0.1	0.2	0.2	948	954
4	benzaldehyde	0.9	0.2	0.2	958	960
5	sabinene	tr	tr	0.2	977	975
6	β-pinene	0.3	0.3	0.4	980	979
7	β-myrcene	tr	0.2	0.3	992	990
8	α-phellandrene	/	0.2	0.6	1004	1002
9	δ-3-carene	/	/	tr	1009	1011
10	α-terpinene	/	0.4	0.1	1016	1017
11	*p*-cymene	0.3	/	/	1023	1024
12	*o*-cymene	/	2.2	1.7	1026	1026
13	α-limonene	0.7	1.0	1.1	1028	1029
14	1,8-cineole	2.3	2.9	3.0	1033	1031
15	salicylic aldehyde	0.4	/	/	1043	1044
16	(*E*)-β-ocimene	/	/	tr	1047	1050
17	γ-terpinene	0.2	0.3	0.3	1060	1059
18	acetophenone	tr	/	/	1063	1065
19	α-terpinolene	tr	0.1	tr	1088	1088
20	linalool	/	2.1	2.5	1098	1096
21	α-thujone	tr	/	/	1101	1102
22	phenyl ethyl alcohol	0.7	/	/	1110	1108
23	camphor	0.2	tr	tr	1148	1146
24	benzyl acetate	/	tr	tr	1160	1162
25	iso-menthone	/	/	tr	1162	1162
26	benzenepropanal	0.5	/	/	1165	1163
27	borneol	tr	tr	/	1170	1169
28	4-terpinenol	tr	0.3	0.2	1178	1177

Table 1. *Cont.*

No.	Compound [a]	EO1 [b]	Sample EO2 [c] % [g]	EO3 [d]	RI [e] (calc.)	RI [f] (lit.)
29	*p*-cymen-8-ol	/	/	tr	1183	1182
30	α-terpineol	tr	0.6	0.3	1189	1188
31	methyl salicylate	tr	/	/	1190	1191
32	2-allyl-phenol	/	/	tr	1193	1191
33	2-methoxy-benzaldehyde	0.5	/	/	1243	1245
34	linalool acetate	tr	/	/	1255	1257
35	2-phenyl ethyl acetate	tr	/	/	1258	1258
36	(*E*)-cinnamaldehyde	77.1	44.1	1.7	1269	1270
37	safrole	/	1.1	1.1	1289	1287
38	geranyl formate	/	0.2	/	1299	1298
39	carvacrol	/	0.1	tr	1302	1299
40	(*E*)-cinnamyl alcohol	tr	0.4	tr	1303	1304
41	α-cubebene	0.4	/	/	1353	1351
42	eugenol	/	23.5	70.8	1360	1359
43	α-ylangene	/	0.9	0.6	1379	1373
44	(*Z*)-caryophyllene	/	0.2	/	1415	1408
45	(*E*)-caryophyllene	tr	3.9	3.5	1422	1419
46	1,2-benzopyrone	2.2	/	/	1437	1434
47	(*E*)-cinnamyl acetate	3.0	3.0	1.5	1449	1446
48	(*E*)-cinnamic acid	tr	/	/	1452	1454
49	α-humulene	/	1.2	/	1456	1454
50	allo-aromadendrene	tr	/	/	1465	1460
51	α-curcumene	tr	/	/	1482	1480
52	α-amorphene	tr	/	/	1485	1484
53	ledene	/	0.2	/	1498	1496
54	α-selinene	/	/	0.6	1499	1498
55	α-muurolene	tr	0.2	/	1504	1500
56	β-bisabolene	tr	/	/	1507	1505
57	eugenol acetate	/	0.5	2.1	1519	1522
58	δ-cadinene	/	0.4	tr	1525	1523
59	(*E*)-*o*-methoxy cinnamaldehyde	8.5	0.3	/	1529	1528
60	caryophyllene oxide	/	0.9	0.4	1583	1583
61	tetradecanal	/	0.1	/	1611	1612
62	benzyl benzoate	/	1.8	3.9	1755	1760
	total	98.9	94.8	99.1		

[a] Identified compounds; [b] CCEO—*Cinnamomum cassia* bark essential oil; [c] CVBEO—*Cinnamomum verum* bark essential oil; [d] CVLEO—*Cinnamomum verum* leaf essential oil; [e] Calculated values for retention indices; [f] Literature values for retention indices; [g] %—percentage amount of identified compounds; [h] tr—compounds identified in amounts less than 0.1 %; [j] /—non-detected, lit.—literature; calc.—calculated.

Table 2. Individual chemical class of identified compounds.

Class of Compounds	EO1 [a]	EO2 [b]	EO3 [c]
	% (Number of Compounds)		
nonterpenic compounds			
aldehydes	/ [d] (0)	0.1 (1)	/(0)
aromatic compounds	93.8 (14)	50.9 (8)	8.4 (8)
subtotal	93.8 (14)	51.0 (9)	8.4 (8)
monoterpenes			
monoterpene hydrocarbons	2.2 (10)	5.7 (11)	6.7 (14)
summ	2.2 (10)	5.7 (11)	6.7 (14)
oxygenated monoterpenes			
monoterpene alcohols	tr (3)	3.1 (5)	3.0 (5)
monoterpene aldehydes	/(0)	0.2 (1)	/(0)

Table 2. *Cont.*

Class of Compounds	EO1 [a]	EO2 [b]	EO3 [c]
	% (Number of Compounds)		
monoterpene ketones	0.2 (2)	tr (1)	tr (2)
monoterpene esters	tr (1)	/(0)	/(0)
monoterpene epoxides	2.3 (1)	2.9 (1)	3.0 (1)
summ	2.5 (7)	6.2 (8)	6.0 (8)
subtotal	4.7 (17)	11.9 (19)	12.7 (22)
phenylpropanoids	/(0)	24.0 (2)	72.9 (2)
subtotal	/(0)	24.0 (2)	72.9 (2)
sesquiterpenes			
sesquiterpene hydrocarbons	0.4 (7)	7.0 (7)	4.7 (4)
summ	0.4 (7)	7.0 (7)	4.7 (4)
oxygenated sesquiterpenes			
sesquiterpene epoxides	/(0)	0.9 (1)	0.4 (1)
summ	/(0)	0.9 (1)	0.4 (1)
subtotal	0.4	7.9 (8)	5.1 (5)
total	98.9 (38)	94.8 (38)	99.1 (37)

[a] CCEO—*Cinnamomum cassia* bark essential oil; [b] CVBEO—*Cinnamomum verum* bark essential oil; [c] CVLEO—*Cinnamomum verum* leaf essential oil; [d] /—nondetected.

3.2. Antioxidant Activity of EOs

Table 3 shows the values for AA from which it can be noted that the analyzed EOs displayed moderate to strong AA. Additionally, the results indicate the strongest AA for the CVLEO (488.0 ± 1.2 TEAC; 84.0 ± 0.3%) which significantly ($p < 0.05$) differed from the other ones. On the contrary, the significantly lowest value for AA was determined in the CCEO (229.0 ± 3.0 TEAC; 29.9 ± 0.6%) among the EOs investigated.

Table 3. Antioxidant activity of analyzed EOs.

EOS	AA (%)	AA (TEAC)
CCEO	29.9 ± 0.6 [a]	229.0 ± 3.0 [a]
CVBEO	82.4 ± 0.1 [b]	480.0 ± 0.5 [b]
CVLEO	84.0 ± 0.3 [c]	488.0 ± 1.2 [c]

Mean ± standard deviation. CCEO—*Cinnamomum cassia* bark essential oil; CVBEO—*Cinnamomum verum* bark essential oil; CVLEO—*Cinnamomum verum* leaf essential oil; AA—antioxidant activity. Values with different superscripts within the same column are significantly different ($p < 0.05$).

3.3. Antifungal Properties of EOs in In Vitro Conditions

In this part of our study, a disc diffusion technique was used to determine the anti-penicillium activities of our analyzed CEOs (CCEO, CVBEO, CVLEO) against fungi strains. As shown in Table 4, the inhibition of fungal growth depended on the kind and concentration of the CEO tested ($p < 0.05$). In effect, with ascending concentrations, the anti-penicillium activities of all EOs increased. Moderate antifungal effectiveness was observed for all analyzed EOs in the 500 µL/L concentration against *P. crustosum* and *P. citrinum*. On the other hand, this concentration of the EOs showed only weak antifungal activity against *P. expansum*. For this *Penicillium* strain, very weak or no antifungal effects were reported for lower concentrations (≤250 µL/L) of the EOs. In the case of *P. crustosum* and *P. citrinum*, very weak growth inhibitory actions were detected for the lowest EO concentrations (62.5 µL/L); the concentrations of 125 and 250 µL/L exhibited very weak activities concerning growth suppression of the fungal strains.

Table 4. *In vitro* anti-penicillium properties of analyzed CEOs in four concentrations (zone of inhibition in mm).

Con. (µL/L)	*P. expansum*				*P. citrinum*				*P. crustosum*			
	62.5	**125**	**250**	**500**	**62.5**	**125**	**250**	**500**	**62.5**	**125**	**250**	**500**
CCEO	0.00 ± 0.00 ^{aA}	0.67 ± 0.58 ^{aB}	2.67 ± 1.15 ^{aC}	6.67 ± 0.58 ^{aD}	2.33 ± 0.58 ^{aA}	5.67 ± 1.15 ^{aB}	8.00 ± 1.00 ^{aC}	10.67 ± 0.58 ^{aD}	3.33 ± 0.58 ^{aA}	6.67 ± 0.58 ^{aB}	8.67 ± 0.58 ^{aC}	14.67 ± 1.15 ^{aD}
CVBEO	0.00 ± 0.00 ^{aA}	1.33 ± 0.58 ^{aB}	4.67 ± 1.15 ^{aC}	8.33 ± 0.58 ^{aD}	1.67 ± 1.15 ^{aA}	5.33 ± 0.58 ^{aB}	5.67 ± 1.15 ^{bB}	11.00 ± 1.73 ^{abC}	3.33 ± 1.15 ^{aA}	6.00 ± 1.73 ^{aB}	9.00 ± 1.00 ^{aC}	13.00 ± 1.73 ^{aD}
CVLEO	0.00 ± 0.00 ^{aA}	0.00 ± 0.00 ^{bA}	3.33 ± 1.15 ^{aB}	8.00 ± 1.00 ^{bC}	1.67 ± 0.58 ^{aA}	6.33 ± 1.15 ^{aB}	7.33 ± 0.58 ^{abB}	12.33 ± 0.58 ^{bC}	4.00 ± 1.73 ^{aA}	5.67 ± 2.08 ^{aA}	9.67 ± 1.53 ^{aB}	14.33 ± 1.15 ^{aC}

Mean ± standard deviation. CCEO—*Cinnamomum cassia* bark essential oil; CVBEO—*Cinnamomum verum* bark essential oil; CVLEO—*Cinnamomum verum* leaf essential oil. Values in the same column with different small letters, and those in the same row with different upper-case letters are significantly different ($p < 0.05$). Con.—concentration; 0.00—total growth.

3.4. Technological Properties of Food Model

Bread slices as a substrate for the growth of fungi displayed values for MC and a_w to be 40.89 ± 0.88% and 0.946 ± 0.002, respectively.

3.5. Microbiological Characterization of Food Model

Our results showed that the number of individual types of microorganisms increased significantly ($p < 0.05$) depending on the duration of bread storage (Table 5). The total count of microorganisms ranged from 0.00 (first day and first week) to 3.04 log CFU/g. Coliform bacteria did not occur in the bread samples during the entire storage period. Microscopic filamentous fungi ranged from 0.00 (first day and first week) to 2.86 log CFU/g.

Table 5. The number of isolated groups of microorganisms in log CFU/g.

Isolated Microorganisms	1st Day	1st Week	2nd Week	3rd Week	4th Week
Total count of microorganisms	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	2.71 ± 0.03 ^{b}	2.87 ± 0.05 ^{c}	3.04 ± 0.02 ^{d}
Coliforms bacteria	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
Microscopic filamentous fungi	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	2.58 ± 0.04 ^{b}	2.77 ± 0.03 ^{c}	2.86 ± 0.04 ^{d}

Mean ± standard deviation. Values in the same column with different small letters are significantly different ($p < 0.05$).

All isolated species from the bread loaves are shown in Table 6. It can be concluded that a total of 41 isolates with high scores were identified in the bread samples. Together seven species of microorganisms were isolated, including *Penicillium* spp. (14 isolates) and *Staphylococcus pasteuri* (13 isolates) as the most isolated ones. Moreover, it can be seen that the isolated species belong to four genera and four families (Table 7).

Figure 1 shows the percentage of isolated species. The most isolated species were *Penicillium* spp. (34%) and *Staphylococcus pasteuri* (32%).

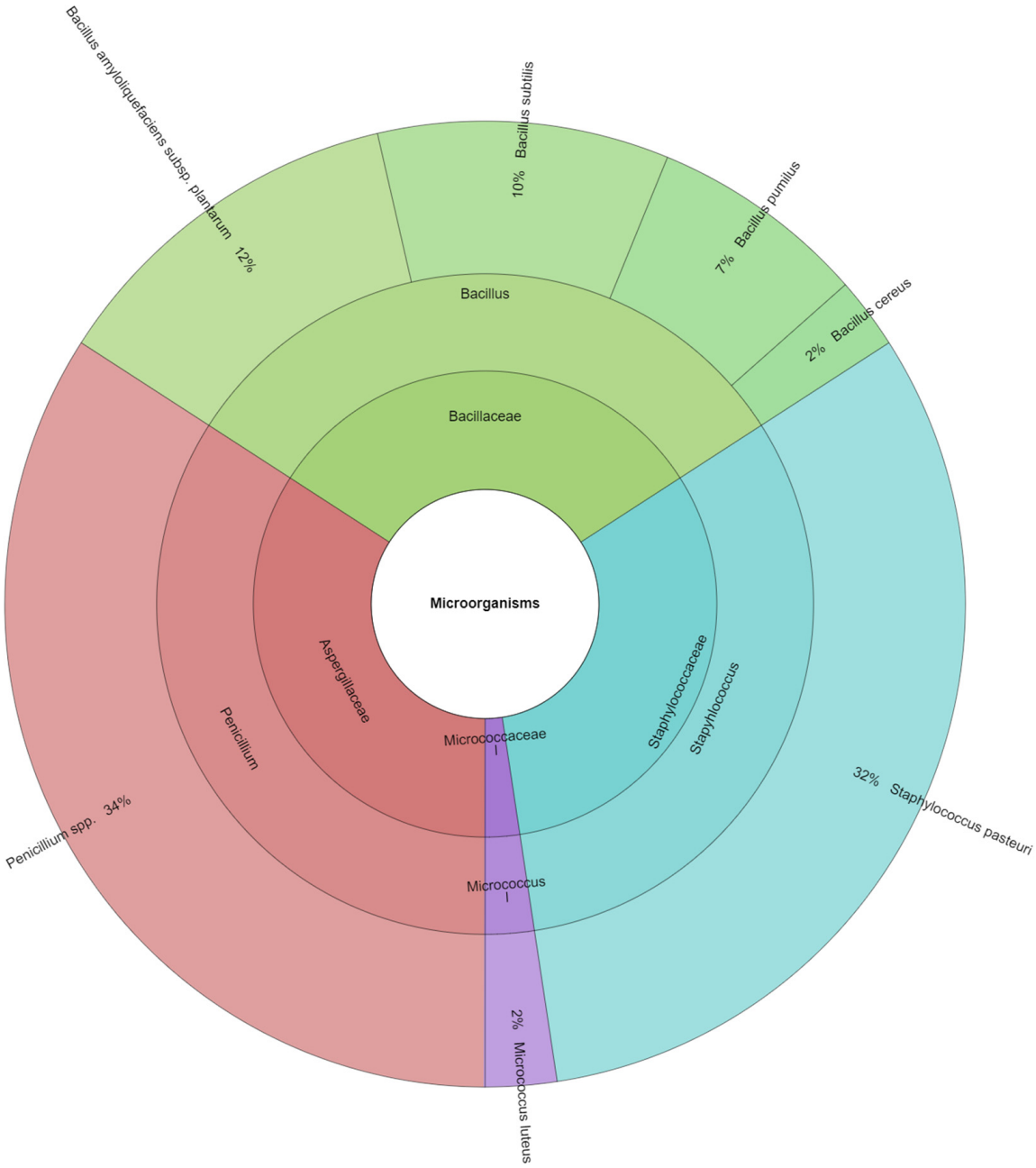

Figure 1. Percentage of isolated species of microorganisms during bread storage.

Table 6. The number of isolated species from bread samples.

Isolated Species	2nd Week	3rd Week	4th Week	Total
Bacillus amyloliquefaciens subsp. *plantarum*		5		5
Bacillus cereus	1			1
Bacillus pumilus		2	1	3
Bacillus subtilis		3	1	4
Micrococcus luteus			1	1
Staphylococcus pasteuri	7	1	5	13
Penicillium spp.	4	5	5	14
Total	12	16	13	41

Table 7. Isolated species of bacteria from bread samples classified into genera and families.

Isolated Species	Genera	Family
Bacillus amyloliquefaciens subsp. *plantarum*	*Bacillus*	Bacillaceae
Bacillus cereus	*Bacillus*	Bacillaceae
Bacillus pumilus	*Bacillus*	Bacillaceae
Bacillus subtilis	*Bacillus*	Bacillaceae
Micrococcus luteus	*Micrococcus*	Micrococcaceae
Staphylococcus pasteuri	*Staphylococcus*	Staphylococcaceae
Penicillium spp.	*Penicillium*	Aspergillaceae

3.6. Antifungal Properties of EOs in In-Situ Conditions

Our data from the vapor contact method (*in situ* analysis) has revealed that the anti-penicillium activity of the EOs obtained from *Cinnamomum* spp. is not only dose-dependent but is also affected by species and the plant part being used for extraction (Table 8). The most effective growth inhibitors were found to be the 250 µL/L of CVBEO and 125 µL/L of CVLEO which almost completely inhibited *P. citrinum* inoculated on bread slices. The mycelial growth of *P. crustosum* was the most inhibited by the 500 µL concentration of CCEO and CVBEO, and *P. expansum* growing on bread was the most sensitive against the action of the 125 and 250 µL/L of CCEO, and 250 µL/L of CVBEO. Contrary, the CVLEO was the least effective against the growth of *P. crustosum* among the EOs investigated. Moreover, its concentration of 250 µL/L was shown to have an even stimulative effect on the growth of the filamentous fungus. The profungal activity was also displayed by the lowest concentration of CVBEO in the case of *P. expansum*.

Table 8. Growth inhibition of mycelium of CEOs.

Fungi Strains	MGI [%] CCEO (µL/L)				CVBEO (µL/L)				CVLEO (µL/L)			
	62.5	**125**	**250**	**500**	**62.5**	**125**	**250**	**500**	**62.5**	**125**	**250**	**500**
P. crustosum	11.90 ± 1.83 ^{aA}	47.25 ± 8.96 ^{bA}	72.32 ± 6.82 ^{cA}	85.63 ± 4.19 ^{dA}	60.13 ± 2.19 ^{aB}	50.95 ± 3.12 ^{bA}	74.13 ± 5.71 ^{cA}	88.00 ± 9.29 ^{cA}	14.74 ± 5.44 ^{aA}	11.49 ± 2.37 ^{aB}	−41.60 ± 3.52 ^{bB}	33.22 ± 9.14 ^{cB}
P. citrinum	49.14 ± 3.69 ^{aA}	67.43 ± 9.72 ^{bA}	85.01 ± 6.57 ^{cA}	68.04 ± 2.32 ^{bA}	33.27 ± 8.79 ^{aB}	48.43 ± 10.59 ^{acA}	95.23 ± 9.17 ^{bA}	57.54 ± 5.41 ^{cB}	23.01 ± 3.16 ^{aB}	96.78 ± 8.54 ^{bB}	71.21 ± 5.36 ^{cB}	39.30 ± 5.79 ^{dC}
P. expansum	48.01 ± 7.51 ^{aA}	82.71 ± 8.62 ^{bA}	80.82 ± 9.56 ^{bA}	69.72 ± 9.19 ^{bA}	−116.10 ± 10.75 ^{aB}	60.97 ± 8.78 ^{bB}	84.01 ± 6.96 ^{cA}	60.67 ± 8.63 ^{bdA}	5.78 ± 1.49 ^{aC}	24.36 ± 6.89 ^{bC}	26.86 ± 2.85 ^{bB}	69.02 ± 7.31 ^{cA}

Mean ± standard deviation. CCEO—*Cinnamomum cassia* bark essential oil; CVBEO—*Cinnamomum verum* bark essential oil; CVLEO—*Cinnamomum verum* leaf essential oil. Values in the same column with different small letters, and those in the same row with different upper-case letters are significantly different ($p < 0.05$). MGI—mycelial growth inhibition.

4. Discussion

The chemical profile of plant EOs may be influenced by various factors including extraction technique, plant issue, cultivar, plant tissue, environment, and geographical origin. Moreover, knowing this parameter is critical to understand EOs biological activities [49]. Among them, the compounds from EOs determine their antioxidant properties [50] and antifungal activity [51], as well. Therefore, chromatographic methods are a suitable tool for understanding the effectiveness of these volatile substances from plants. In agreement with our findings, Li et al. [52] analyzed the major components of EOs isolated from *C. cassia* bark to be (*E*)-cinnamaldehyde (69.75%), and 2-methoxycinnamaldehyde (5.92%). Also, Chahbi et al. [38] identified (*E*)-cinnamaldehyde (69.15%) as the main compound of CCEO used in their study. Similarly, we have found out that the major component in the conception of CVBEO was (*E*)-cinnamaldehyde (44.1%). In line with these findings, several researchers have stated that cinnamaldehyde is the main volatile substance of *C. verum* bark essential oil [53–56]. On the other hand, it is known that the most ordinary chemotype found in the EO of *C. verum* leaf is eugenol [57] corresponding with our findings (70.8%). Based on our analysis we can conclude that the presence of individual components in EOs depends not only on the *Cinnamomum* species, but also on the extracted part of the plant.

In general, DPPH testing has been used to evaluate the antioxidant properties of various food products [58,59]. DPPH radical is a very stable nitrogen-centered radical, which can be applied to evaluate the free radical scavenging ability relating to their antioxidant efficacy [60]. Thus, the above-mentioned technique deals with the measurement of DPPH concentration changes with a spectrophotometer resulting from its reaction with an antioxidant [61]. Generally, it is known that the AA of EOs can vary depending on their chemical profile [62]. Both the minor and major chemical compounds can affect significantly the AA of EOs; therefore, it is not easy to attribute the AA of the EO to only some of its components [63]. This view is also confirmed by our results, where (*E*)-cinnamaldehyde as the main substance in both CCEO (77.1%) and CVBEO (44.1%) chemical conceptions was detected; however, the AA of these oils was very different (29.9 $\pm$ 0.6%, and 82.4 $\pm$ 0.1%, respectively). On the other hand, in our CVLEO (exhibiting the highest AA among all three EOs analyzed) as the main compound was found to be eugenol (70.8%), which is known as a substance with proven antioxidant characteristics [64]. Furthermore, Castañeda et al. [65] determined in ethanolic extracts of *C. verum* very high values for AA with a percentage of inhibition of 97.59%, which corresponds to our results (more than 82.4% inhibition exhibited by our EOs isolated from *C. verum* bark and leaf). In addition, a comparable value for AA of *C. cassia* bark EO (335.78 $\pm$ 77.15 TEAC) with the data obtained from our CCEO (229.0 $\pm$ 3.0 TEAC) was reported by Yang et al. [66]. From these results, we concluded that AAs of EOs are on a large scale influenced by the species of plants [67], as well as their extracted parts [68].

The antimicrobial characteristics of diverse EOs isolated from plants are well-known since ancient times [69], and at present, more and more scientists have been focused on determining their ability to eliminate the presence of various foodborne pathogens [70]. Indeed, various types of research papers reveal antifungal activities of diverse EOs against a large scale of fungal strains, including *Penicillium* spp. (*P. roqueforti*, *P. solitum*, *P. echinulatum*, *P. commune*, *P. camemberti*, *P. polonicum*) [71].

El-Baroty et al. [72] evaluated the inhibitory effect of *C. verum* bark EO (in four concentrations: 10, 25, 50, 75, and 100 µg/mL) on the growth of four fungal strains, *Aspergillus niger*, *P. notatum*, *Mycena hiemalis*, and *Fusarium oxysporum*. In accordance with our study, the authors noted a dose-dependent effectiveness of the oil, whose highest concentration (100 µg/mL) induced up to 100% growth inhibition of all evaluated strains. Low values for minimum inhibitory concentration (MIC) of *C. verum* bark EO (0.1563 µL/mL) against *P. corylophilum* (declaring its antifungal efficacy) have been observed in the study performed by Ji et al. [73]. The antifungal potential of cinnamon leaf EO against *Eurotium*, *Aspergillus*, and *Penicillium* spp. has also been shown by the results of Guynot et al. [74]. Many scientific reports have documented that the antifungal properties of EOs can be connected to the

attendance of individual bioactive substances present in their composition [7,15]. Therefore, we assume that (*E*)-cinnamaldehyde may be responsible for the antifungal activities of our EOs (mainly CCEO, and CVBEO) which was also proven by Shreaz et al. [75] whose findings revealed the substance to be effective against the growth of yeasts, bacteria, and microscopic filamentous fungi. Moreover, the inhibitory effects and mechanism of the substance's action on fungi growth and mycotoxin production have been demonstrated by Liang et al. [76]. In this study, the growth of *A. flavus* and aflatoxin B1 (AFB1) generation were completely inhibited by 0.80 mmol/L of cinnamaldehyde. Its lower concentration (0.40 mmol/L) markedly decreased AFB1 production with an inhibition scale of 68.9%, but it had no effect on fungal growth showing a dose-dependent effect. The inhibitory action of (*E*)-cinnamaldehyde against microorganism growth is based on the inhibition of ATPases [77] biosynthesis of their cell walls [78], and changes in their membrane structure and integrity [79]. In addition, the antifungal effects observed in our study may also be attributed to the high content of eugenol (the main compound in our CCVEO) which was evaluated in some previous reports dealing with the prevention of fungal spore germination and mycelial growth [80–82]. Mechanisms of eugenol action are associated with its destructive effects on the envelope of fungal cells [83], and with disruption of the cytoplasmic membrane due to the ability of its phenolic hydroxyl group to increase eugenol solubility in aqueous suspensions that improve its transition through the hydrophilic section of the cell envelope [84].

Since wheat bread is a staple food [85], we used it as a model food for *in situ* evaluation of the antifungal efficacy of the EOs in the next part of our study. Day et al. [2] stated that this bakery product belongs to an intermediate-moisture food with MC ranging from 35 to 42% and value for a_w above 0.95, which is in agreement with our results (40.89 $\pm$ 0.88% and 0.946 $\pm$ 0.002, respectively). Moreover, it is known that bread loaves are prone to mold spoilage after a few days of storage [3] which corresponds with our results demonstrating the growth of total microorganisms and fungi on our bread samples after two weeks of their storage period. Most importantly, the presence of coliform bacteria was not detected in our bread samples during the entire period of storage reflecting the safety of the bread and its correct production technology [86]. Among microorganisms detected in our study, the most isolated species was *Penicillium* spp. which is in line with the results of Melini and Melini [4] recognizing *Penicillium* as the most common kind of bread mold. Summarily, wheat bread is susceptible to microbial spoilage, suggesting its application as a suitable model substrate for similar kinds of experiments.

The antimicrobial properties of diverse EOs have been intensively studied against several organisms *in situ*, commonly by direct contact methods [87,88]. However, these methods may be less successful due to the high hydrophobicity or volatility of EOs, altering their antimicrobial effectiveness [89]. Contrastly, vapor phase techniques propose more credible results concerning the effectiveness assessment of these volatile substances present in EOs [90]. Hence, vapor phase contact of EOs as antimicrobial substances may have applications, particularly for microscopic filamentous fungi, which normally grow and spoil food surfaces [91]. Another benefit of this application is the fact that the vapor phase method of EOs decreases their impact on the sensory properties (aroma) of evaluated products in comparison with EOs added directly to them [92]. Confirming these findings, our previous studies demonstrated a preferable activity of different types of EOs (lavender, mint, rosemary, lemongrass, fir, rosalina, niaouli) in the vapor phase against *Penicillium* spp. [45,46,93]. The efficacy of treatment of our bread with three types of CEOs in our study is in agreement with Clemente et al. [94], who observed an effective reduction of incidence and development of natural mold spoilage (isolated from whole wheat bread in natural conditions) of wheat bread loaves stored during ten days. Moreover, the antifungal characteristics of EOs applied in the current study and their dose-dependent effectiveness, as well as differences between their individual types support our previous research focused on the investigation of *in situ* antifungal efficacies of other EOs, such as

Cymbopogon citratus [46], *Malelauca* (*M.*) *armillaris* subsp. *armillaris*, *M. quinquenervia*, and *Abies alba* [45] against the *Penicillium* strains growing on food models.

In summary, the CCEO, CVBEO, and CVLEO appear to be promising antifungal substances increasing the shelf-life of wheat bread. In order to eliminate the negative sensory properties of the final products, we intend to carry out an evaluation of the organoleptic properties of the treated bread samples in the future.

5. Conclusions

The present research was designed to analyze the chemical profile, AA, and antifungal properties of three types of commercial EOs including CCEO, CVBEO, and CVLEO against *Penicillium* spp. Our results revealed a variable chemical composition of the investigated EO samples with (*E*)-cinnamaldehyde (CCEO and CVBEO), and eugenol (CVLEO) being major substances of their chemical profile. Among the EOs, the strongest AA was detected for the CVLEO (488.0 ± 1.2 TEAC; 84.0 ± 0.3%) which was significantly ($p < 0.05$) different. The strongest antifungal efficacies (*in vitro* experiments) were displayed by all analyzed EOs in the 500 µL/L concentration against *P. crustosum*, which inhibition zones ranged from 13.00 ± 1.73 mm (CVBEO) to 14.67 ± 1.15 mm (CCEO). The findings from the evaluation of MC, a_w, and microbial characterization of the wheat bread showed excellent growth possibilities for fungi. Realized vapor contact method displayed dose-dependent antifungal actions of all EOs with the highest MGI in 250 µL/L of the CVBEO against *P. citrinum* (95.23 ± 9.17%). Finally, our findings suggest that the CCEO, CVBEO, and CVLEO can be used as novel natural substances for the shelf-life prolongation of wheat bread. Moreover, the data complement our previous reports, thereby contributing to the creation of a more complete report dealing with the biological characteristics of various commercial EOs obtained from the same company (Hanus Ltd., Nitra, Slovakia).

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