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Original article

Antimicrobial effect of cinnamon (*Cinnamomum verum* J. Presl) bark essential oil in cream-filled cakes and pastries

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Abstract

Background and objectives: Food poisoning has been always a major concern in health system of every community and cream-filled products are one of the most widespread food poisoning causes in humans. In present study, we examined the preservative effect of the cinnamon oil in cream-filled cakes. **Methods:** Antimicrobial activity of *Cinnamomum verum* J. Presl (Cinnamon) bark essential oil was examined against five food-borne pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Bacillus cereus* and *Salmonella typhimurium*) to investigate its potential for use as a natural preservative in cream-filled baked goods. Chemical constituents of the oil were determined by gas chromatography/mass spectrometry. For evaluation of preservative sufficiency of the oil, pathogens were added to cream-filled cakes manually and 1 µL/mL of the essential oil was added to all samples except the blank. **Results:** Chemical constituents of the oil were determined by gas chromatography/mass spectrometry and twenty five components were identified where cinnamaldehyde (79.73%), linalool (4.08%), cinnamaldehyde para-methoxy (2.66%), eugenol (2.37%) and trans-caryophyllene (2.05%) were the major constituents. Cinnamon essential oil showed strong antimicrobial activity against selected pathogens *in vitro* and the minimum inhibitory concentration values against all tested microorganisms were determined as 0.5 µL/disc except for *S. aureus* for which, the oil was not effective in tested concentrations. After baking, no observable microorganism was observed in all susceptible microorganisms count in 72h stored samples. **Conclusion:** It was concluded that by analysing the sensory quality of the preserved food, cinnamon oil may be considered as a natural preservative in food industry, especially for cream-filled cakes and pastries.

Keywords: antimicrobial, *Cinnamomum verum*, essential oil, food-borne pathogens, preservative

Introduction

Food poisoning affects many people around the world every year and cream-filled products are one of the most widespread food poisoning causes in humans. The main causes of

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contamination among these products are well-known pathogens including *Escherichia coli* and *Salmonella typhimurium* [1]. As community demands for safe and natural products have increased, introduction of new methods for preservation of foods by evaluation of new natural sources has dramatically risen. Volatile oils are one of the most important antimicrobial constituents of medicinal herbs which mainly comprise from terpenoids [2]. They are considered as new alternative candidates which may be employed as preservatives in future [3].

Cinnamomum verum J. Presl (Lauraceae family), commonly known as cinnamon, grows mainly in South and South-East Asia and its bark is rich in essential oil (EO) with a potent antimicrobial activity [4]. Although the oil from different parts of the world has shown great diversity in chemical composition [5-7], it comprises mainly of trans-cinnamaldehyde as the major component (47-71%). The strong antibacterial and antifungal activity of the *C. verum* EO has been reported in different studies, alone [4, 8-9] or in combination with other essential oils [4]; however, it has failed to show any antimicrobial activity when used in yogurt in concentration of 0.04% which was corresponded to the highest acceptable concentration established during sensory acceptance assays [3]. In order to control the growth of possible food contaminants and extending the shelf life of cream-filled cakes and pastries, we examined the preservative effect of the cinnamon oil (which has been considered by US Food and Drug Administration "generally recognized as safe") [10] in this type of foods.

Exprimental

Essential oil preparation

The essential oil was purchased from Zardband Company, Tehran, Iran, in May 2015 and kept at 2-8 °C in sealed brown vials until required.

Determination of essential oil chemical composition

Chemical composition of the EO was determined by Gas chromatography (GC)/ GC-MS (mass spectrometry) instruments.

Termoquest 2000 gas chromatograph. Column: capillary column HP-1MS, 30 m-0.25 mm-0.25 μm film thickness; temperature program: from 40 ° to 250 °C (60 minutes) at 3 °C/minute and then kept constant at 250 °C for 60 minutes. Injection temperature: 250°C. Injection volume: 1.0 μL, carrier gas: He, injection mode: split (25:1).

GC/GC-MS was performed with a Thermoquest 2000 with a quadruple detector. Column: capillary column HP-1MS, 30 m-0.25 mm- 0.25 μm film thickness; temperature program: from 40 °C (1 minute) to 250°C (60 minutes) at 3°C/minute and then kept constant at 250 °C for 60 minutes. Injection temperature: 250 °C. Injection volume: 1.0 μL, carrier gas: He, injection mode: split (25:1). MS interface temp.: 250 °C; MS mode: EI; detector voltage: 70 eV. Retention indices were calculated by using retention times of standard normal alkanes (C_6–C_{27}) that were injected after the oil at the same chromatographic conditions.

Quantitative data were obtained from the electronic integration of the flame ionization detector (FID) peak areas. The components of the oil were identified by comparison of their mass spectra and retention indices with the published data in references [11–14].

Antibacterial activity of cinnamon essential oil

The antimicrobial activity of the cinnamon essential oil was evaluated by the disc diffusion method against five main food-borne pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Bacillus cereus* and *Salmonella typhimurium*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the oil were assessed by the broth microdilution method with visible growth observed by using 96 U-shaped-well plates, according to our previous study [1].

For disc diffusion assay, Petri dishes with Mueller–Hinton agar (for bacteria) or Sabouraud Dextrose agar (for the yeast) were seeded with a test strain suspension (1.5×10^8 cfu/mL) using a sterile cotton swab. Then, filter paper discs (6

mm in diameter) were impregnated with 05, 1, 2, 4, 8, 16 and 32 µL of cinnamon oil and placed on the inoculated plates, which were incubated at 37 °C for 24 h for bacterial strains and 35 °C for 48 h for the yeast. Inhibition was detected by measuring clear zones around the discs in millimeters. The lowest concentration of the essential oil showing a clear zone of growth inhibition around the disc was used to obtain the MIC.

For MIC determination, a stock concentration of 10 µL/mL from the essential oil in Mueller-Hinton/Sabouraud Dextrose broth (Merck Co. Germany) was prepared which was subjected to twofold serial dilutions. Then a 100 µL aliquot of the twofold test strain inoculum was added to each well to reach the final inoculum amount of 5×10^5 cfu/mL. After proper incubation, the microdilution plates were examined for visible growth of microorganisms in comparison with the essential oil-free well (control). MIC was recorded as the lowest concentration of the oil at which the microorganisms did not show any visible growth. The MBC was determined by quantitative subculture of 100 µL from each clear well into MH/SD agar plates. The plates were incubated at respective temperature and time. The MBC was defined as the lowest concentration of the essential oil that resulted in more than 99.9% killing of the tested microorganism.

Evaluation of essential oil antimicrobial activity in cream-filled cake

The purchased cream and pastes were used for baking cream-filled cakes, according to our previous published work [1]. One milliliter of each of the four susceptible food-borne pathogens (obtained in the previous section, containing 1.5×10^5 cfu/cake) was added separately to beakers containing the cake samples. Then cinnamon essential oil was added (1 µL/g of cake) to 9 beakers. Three beakers were used as blanks (without essential oil). The entire mixtures were then heated in 120 °C for 10 min and then kept in refrigerator. Total microbial counts were obtained with the pour-plate method at time zero and 72 h (expected shelf-life for cakes in confectionaries) after baking, and were compared with blank.

The cream part of each sample was separately added to sterile 0.1% peptone, homogenized in a stomacher for 2 minutes and diluted serially in 0.1% peptone solution. One hundred-microliter aliquots of serial dilutions were spread-plated in triplicate on the surface of Tryptic soy agar (TSA; Merck, Germany) incubated at 37°C for total bacterial count and Sabouraud dextrose agar (SDA; Merck, Germany) incubated at 35 °C for total fungal count. After 48–72 hours, the average number of visible colonies obtained from plate counts were determined and transformed to log.

Results and discussion

The essential oil of *C. verum* bark was yellow in color and had a characteristic odor of cinnamon. Twenty five chemical compounds were identified in the oil, which comprised 98.93% of the total oil. The dominant constituents were trans-cinnamaldehyde (79.73%), linalool (4.08%), cinnamaldehyde para-methoxy (2.66%), eugenol (2.37%) and trans-caryophyllene (2.05%). Phenylpropanoids were the major class of compounds in the essential oil (81.7%) (table 1).

Cinnamon oil exhibited potent antibacterial effect against contaminating microorganisms, *in vitro*. The MIC of the essential oil against the tested microorganisms (except for *S. aureus* in which the oil was ineffective in tested concentrations) was 0.5 µL/disc or µL/mL in disc diffusion and microdilution methods, respectively. The lowest concentration in which more than 99.9% reduction in microbial counts was observed for all microorganisms (except for *S. aureus*) was 1 µL/mL of the essential oil, which was recorded as MBC (table 2).

Pour-plate counts of four main pathogens in baked cream-filled cakes including essential oil at time zero and after 72 hours of baking did not show any observable colonies, whereas the blank samples showed the count of 1.5×10^3 cfu/cake of each pathogen.

Table 1. Chemical composition of *Cinnamomum verum* bark essential oil

No.	Compound	RI_S[a]	RI_I[b]	RT[c]	Percent
1	alpha-Pinene	1086	939	20.68	0.50
2	Camphene	1102	953	21.50	0.08
3	Benzaldehyde	1114	961	22.08	0.31
4	beta-Pinene	1132	980	23.00	0.07
5	beta-Myrcene	1142	991	23.51	0.40
6	1-phellandrene	1159	1005	24.38	0.18
7	Delta-3-carene	1166	1011	24.71	0.37
8	*O*-cymene	1181	1022	25.47	1.31
9	limonene	1185	1031	25.69	0.19
10	beta-Phellandrene	1186	1031	25.76	0.25
11	1,8-cineole	1189	1033	25.90	1.02
12	Benzyl alcohol	1192	1032	26.06	0.14
13	Linalooll	1287	1098	29.38	4.08
14	Phenylethyl alcohol	1308	1110	30.29	0.15
15	*trans*-Cinnamaldehyde	1489	1266	38.96	79.73
16	Eugenol	1552	1356	42.00	2.37
17	alpha-copaene	1570	1376	42.93	0.21
18	*trans*-caryophyllene	1622	1431	45.02	2.05
19	Cinnamyl acetate	1646	1386	45.62	1.48
20	Coumarin	1652	1429	45.79	0.47
21	Alpha-humulene	1677	1454	46.43	0.19
22	Cinnamaldehyde-*p*-methoxy	1756	1561	49.41	2.66
23	Caryophyllene oxide	1812	1581	51.84	0.35
24	Benzyl benzoate	1962	1762	58.29	0.37
25	Unknown	2235	-	70.02	0.73
26	Unknown	2242	-	70.33	0.34
Total identified					98.93
Unknown					1.07
Phenylpropanoids				86.39	
Oxygenated Monoterpens				5.10	
Hydrocarbon Monoterpens				3.35	
Hydrocarbon Sesquiterpenes				2.45	
Simple aromatic compounds				1.29	
Oxygenated Sesquiterpenes				0.35	

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of cinnamon bark essential oil against selected bacteria and the yeast by broth microdilution method

Pathogen	MIC (µL/mL)	MBC (µL/mL)
Escherichia coli	0.5	1
Salmonella typhimurium	0.5	1
Candida albicans	0.5	1
Bacillus cereus	0.5	1
Staphylococcus aureus	-[a]	-

[a] not effective in tested concentrations

According to previous studies on chemical composition and antimicrobial activity of cinnamon bark oil, cinnamaldehyde had been reported as the main component in the majority of the studies [5-7]. Eugenol has been reported to be a major component of cinnamon leaf which should be distinguished from the bark oil [15,16].

It has been shown that enrichment of fresh products with cinnamon oil has been effective in reducing subsequent colonies of specific pathogens, especially fungi [16]. Ojagh *et al*. examined the effect of chitosan coating of rainbow trout, enriched with 1.5% cinnamon oil and concluded that combined coating can enhance the shelf life of frozen fishes [17].

Antimicrobial activity of trans-cinnamaldehyde has been shown already in earlier studies, [18,19]. It is commonly used in food and beverages as a flavouring ingredient [20]. Recently, it has been successfully developed as an antimicrobial preservative film to pack bread (0.0025 g/g) and pastry (0.005 g/g), with a good acceptance of the bread samples [18].

Because of complex matrix of foods, compared to *in vitro* studies, usually higher concentrations are needed in order to inhibit the growth of specific microorganisms [3]. Although Gutierrez *et al*. report about the best criteria for the EOs to be more efficient against pathogenic bacteria in model food media with high protein and low fat and carbohydrate content, [21,22], we observed that cinnamon EO was as effective as *in vitro* experiment in a high fat medium (cream).

Our results demonstrated that the use of *C. verum* bark essential oil in concentrations higher than MIC values (i.e. >0.5 µL/mL) in cream-filled cakes can prolong the time needed for the natural microflora to reach concentrations able to produce a noticeable degradation and reduce the risk of food poisoning due to consumption of contaminated products.

Mortiz *et al*. [3] have determined the higher acceptable sensory concentration of cinnamon oil which was 0.04%. However, a combination of cinnamon oil in higher acceptable sensory concentration (0.04%) and ethylenediaminetetraacetic acid (EDTA) was not been effective in inhibition of aerobic mesophiles, yeasts and molds [3].

In the present study, concentrations more than 0.05% were effective in reduction of microorganisms growth, thus, it must be noted that for being used as a preservative, sensory

quality of the baked cakes and pastries should be analyzed in the future.

According to the results of the present study, cinnamon oil could be a potential natural alternative candidate for cream-filled cakes and pastries preservation.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

EO: Essential oil
US: United States
GC: Gas chromatography
GC-MS: Gas chromatography/Mass spectrometry
FID: Flame ionization detector
MIC: Minimum inhibitory concentration
MBC: Minimum bactericidal concentration
MHB: Mueller–Hinton broth
MH: Mueller–Hinton
TSA: Tryptic soy agar
SDA: Sabouraud dextrose agar
EDTA: Ethylenediaminetetraacetic acid