International Food Research Journal 25(2): 540-544 (April 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effects of oregano, cinnamon, and sweet fennel essential oils and their blends on foodborne microorganisms

[1]Ribeiro-Santos, R., [2]Ventura, L. A. F., [2]Santos, D. C., [1,2,*]Melo, N. R. and [1]Costa, B. S.

[1]Department of Food Technology, Technology Institute, Rural Federal University of Rio de Janeiro, BR485, km 7, PO box 74573, 23890-971, Seropédica, Rio de Janeiro, Brazil
[2]Department of Agribusiness Engineering, Fluminense Federal University, Avenida dos Trabalhadores, 420, CEP 27225-250, Volta Redonda, RJ, Brazil

Article history

Received: 19 January 2017
Received in revised form: 23 February 2017
Accepted: 26 February 2017

Keywords

Food safety
Natural antimicrobials
Foodborne microorganisms
Antimicrobial activity
Commercial essential oils
Synergistic effect

Abstract

Essential oils (EO) have stood out for their potential application as antimicrobial agents, playing an important role in ensuring food quality and safety. In addition, the synergistic effect of EO blends can improve action spectrum for more effective applications. The purpose of this research was to evaluate the *in vitro* antimicrobial activity of oregano, sweet fennel, and cinnamon EOs, and their blends, against the pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*, and the fungus *Penicillium* spp. The antimicrobial activity was determined by the disk-diffusion method, at both microbial optimal growth temperature and refrigeration temperature. Different EO volumes were investigated, and an effective inhibition was observed for the concentration of 3 µL. Oregano EO has proven to be effective in all assays, when compared to the EO combinations.

Introduction

Food is normally susceptible to contamination by several pathogens, including bacteria and fungi. Many studies have shown the effectiveness of various essential oils (EO) against foodborne microorganisms (Viuda-Martos *et al.*, 2012; Dussault, Vu and Lacroix, 2014; Szczepanski and Lipski, 2014). Furthermore, EO extracted from spices has been recognized as GRAS (Generally Recognized as safe) by the FDA (Burt, 2004). There is a growing interest in the industry and scientific community to study herbs and medicinal plants due to their antimicrobial, antioxidant, and aromatic properties (Bakkali *et al.*, 2008).

Essential oils are volatile compounds synthesized in several plant organs, including flowers, buds, seeds, leaves, stems, or bark (Dvaranauskaite *et al.*, 2009; Wannes *et al.*, 2010; Lv *et al.*, 2012; Hill *et al.*, 2013), being , rich in terpenoids and phenolic compounds (Rodríguez *et al.,* 2007), which are responsible for their biological activity. Several antimicrobial agents, including EO, can be used in food or as active materials for food packaging, thus increasing the shelf life and safety of the products, by reducing or preventing growth of pathogenic and spoilage microorganisms.

The synergistic effects of the EO components have been widely studied, which can broaden the action spectrum for more effective applications, besides improving food safety. Thus, EO blends can enhance the antimicrobial effect against a wide range of microorganisms (Lopez *et al.*, 2007). Therefore, the present study aimed to evaluate the antimicrobial effectiveness of cinnamon, oregano, and sweet fennel EOs, alone or in combination, against *E.coli, S. aureus*, and *Penicillium* spp.

Materials and Methods

Organisms and growth conditions

The microbial cultures were obtained from the American Type Culture Collection (ATCC) at Federal University of Viçosa, Minas Gerais, Brazil. The following organisms were studied: the gram-negative strain *Escherichia coli* (ATCC 1122), gram-positive strain *Staphylococcus aureus* (ATCC 6538), and fungal strain of the genus *Penicillium* spp. The microbial cultures were activated in brain heart infusion broth (BHI) for 24h at 36±2°C and 25±2°C for bacteria and fungi, respectively. After, the cultures were incubated in Plate Count Agar (PCA) (bacteria) and Potato-Dextrose-agar (BDA) (fungi), for 48h at 36±2°C, and 5 days at 25±2°C,

*Corresponding author.
Email: *nathaliarm@vm.uff.br*

Table 1.Common name, plant variety, major components, and plant part used to extract the essential oils, according to the supplier

Common name	Plant variety*	Major components*	Plant part *
Cinnamon	*Cinnamomum Zeylanicum* Blume	Eugenol	Leaves
Sweet fennel	*Foeniculum vulgare* Dulce	Anethole	Seeds
Oregano	*Origanum vulgare* L	Carvacrol	Leaves

*Ferquima®

respectively. Inoculum was prepared by diluting cultures in peptone water.

Essential oils

Three commercial essential oils from plants were purchased from Ferquima® (Ferquima Indústria e Comércio Ltda, Vargem Grande Paulista, São Paulo). The characteristics of the EOs, including common name, major components, plant variety, and plant part used in the extraction are presented in Table 1. All EOs selected for this study were produced by distillation, and kept at room temperature in an amber glass bottles.

In vitro *antimicrobial activity*

Agar disk-diffusion method (CLSI 2003) was used to determine the antimicrobial activity of the EOs (cinnamon, sweet fennel, and oregano) against *E.coli, S.aureus,* and *Penicillium* spp.

A 100 µL suspension containing 10^4 CFU/mL of bacteria and 10^4 spores/mL of fungal spores was spread over the surface of Mueller-Hinton agar plates. Then, a sterile paper disk (Whatman, 10 mm in diameter) was placed on the agar surface, and EO was placed at the center of each disk. A sterile paper disk without addition of EO was used as negative control.

Different EO volumes were tested, as follows: 60, 20,15,10,8,5, and 3 µL. The plates inoculated with bacteria and fungal spores were incubated at 35 ±2°C for 48h, and 25 ±2°C for 5 days, respectively. In addition, plates inoculated with bacteria and fungi were both incubated at 8±2°C to simulate the refrigerated storage. After incubation, microbial growth was observed on the plates, and the inhibition zone diameters (colony-free perimeter) were measured with a caliper. Data were expressed as inhibition zones (cm), not including the disk area. Each experiment was performed in triplicate.

Combination of essential oils

The essential oils were blended in equal parts, as follows: oregano + sweet fennel (OF); oregano + cinnamon (OC); cinnamon + sweet fennel (CF); and cinnamon + sweet fennel + oregano (CFO), A volume of 3 µL of EO blends was used in the experiments, once it was the lowest effective dosage as determined in preliminary tests, thus reducing costs with EO.

Statistical analysis

The experiment was carried out in a completely randomized design. All determinations were carried out in three replicates, and the results were expressed as mean of each replicate. Differences between the results were evaluated by analysis of variance (ANOVA) using the F-test, and Tukey's test was used to compare means at 5% probability, using the SAS® software 9.0 (SAS Institute Inc., NC, USA).

Results and Discussion

In general, the oregano EO exhibited the highest antimicrobial effect, followed by cinnamon EO and sweet fennel EO. Biological activities are dependent on the composition of essential oil. Some authors studied different components present in EO and found higher antimicrobial activity in carvacrol followed by eugenol and anethole (major component of sweet fennel EO) (Sleha *et al*., 2014; Wieczynska *et al*., 2016). Studies have also shown that EOs containing phenols as major components, such as carvacrol (major component of oregano EO) or eugenol (major component of cinnamon EO) presented the highest antimicrobial activity (Bassolé, and Juliani, 2012).

Volumes of EO tested: 60 and 20 µL

The volumes of EO of 60 and 20 µL have poured to the culture medium, thus it was not possible to determine the inhibition zones to confirm the antimicrobial effect against the bacteria and fungi for these volumes.

Volumes of EO tested: 5, 10, and 15 µL

The antimicrobial activity of the EO was evaluated using the disc-diffusion method (Table 2). The higher the volume of EO, the greater the inhibition zone, thus the volumes of 10 and 15 µL EO showed higher antimicrobial effectiveness when compared to 5 µL EO.

Both oregano and cinnamon EOs exhibited antimicrobial activity against all microorganisms in the temperatures tested. In general, oregano

Table 2. Mean inhibition zone diameters (cm) for the different dosages (5, 10, and 15 µL) of essential oils against *E. coli, S. aureus*, and *Penicilium* spp. at both optimum and refrigeration temperatures. Disc diameter is not included.

Microorganism	Temperature	Mean inhibition zone diameter (cm)								
		Cinnamon EO			Sweet fennel EO			Oregano EO		
		5 µL	10 µL	15 µL	5 µL	10 µL	15 µL	5 µL	10 µL	15 µL
E. coli	35 ± 2ºC	1.5	-	2.3	n.d.	-	n.d.	3.0	-	5.5
	8 ± 2ºC	0.8	-	2.5	n.d.	-	0.6	6.5	-	9.0*
S. aureus	35 ± 2ºC	1.4	-	1.7	n.d.	-	1.2	6.5	-	9.0*
	8 ± 2ºC	9.0*	-	9.0*	9.0*	-	9.0*	9.0*	-	9.0*
Penicilium spp	25 ± 2ºC	3.4	3.3	-	1.0	8.0*	-	6.4	7.5	-
	8 ± 2ºC	9.0*	9.0*	-	9.0*	9.0*	-	9.0*	9.0*	-

- not studied; n.d. not detected; * No microbial growth; 9 cm Petri dish diameter.

Table 3. Mean inhibition zone diameters (cm) for the antimicrobial activity of 3 µL EOs against *E. coli, S. aureus*, and *Penicilium* spp. Disc diameter is not included.

Microorganism	Essential Oils		
	Cinnamon	Sweet fennel	Oregano
Escherichia coli	0.36^{aA}	0.00^{aB}	2.17^{aC}
Staphylococcus aureus	3.03^{bA}	3.68^{bA}	5.33^{bB}
Penicillium spp.	4.53^{bA}	3.60^{bA}	6.20^{bB}

*Means within columns followed by the same superscript lower case letter, and means within rows followed by the same superscript uppercase letter are not significantly different from each other ($p > 0.05$), by Tukey test.

EO presented the highest effectiveness, followed by cinnamon EO, while the sweet fennel EO was less effective, as can be seen in Table 2. Similar results were observed by Dussault, Vu and Lacroix (2014), who studied the antibacterial properties of commercial essential oils (oregano > cinnamon > sweet fennel).

Refrigeration is probably the most popular form of food preservation, and, in this study, EO in combination with refrigeration temperatures prevented the growth of *S.aureus* and *Penicillium* spp, and decreased growth of *E.coli* at 8 ± 2ºC for10 days. A greater antimicrobial effect was observed against Gram-positive bacteria as *S. aureus,* when compared with the gram-negative strains as *E. coli*, as also reported by several authors (Zinoviadou, Koutsoumanis and Biliaderis, 2009; Viuda-Martos *et al.*, 2012; Teixeira *et al.*, 2013). The lower antimicrobial activity against the gram-negative strains may be due to a higher resistance of these microorganisms provided by the external lipopolysaccharide wall surrounding the peptidoglycan cell wall (Zinoviadou, Koutsoumanis and Biliaderis, 2009). In this study, the cinnamon EO inhibited both the fungal growth and the production of spores, which was also observed by Nielsen and Rios (2000), and Xing *et al.* (2010) for *Aspergillus flavus*.

Volumes of EO tested: 3, 5, and 8 µL

Whereas no significant ($p > 0.05$) differences were observed for lower volumes of EO (3, 5, and 8µL) (data not shown), the lowest effective volume (3 µL) was used throughout the experiment, once similar results can be achieved at lower costs. Oregano EO was more effective against the microorganisms evaluated, followed by cinnamon EO and sweet fennel EO (Table 3), with no significant differences ($p > 0.05$) between cinnamon and sweet fennel essential oils against *S. aureus* and *Penicillium* spp.. All EO exhibited low antimicrobial activity against *E.coli,* except sweet fennel EO that had no activity against this microorganism. Souza and others (2013) and Goñi and others (2009) studied the inhibition of *Aspergillus flavus* and *Aspergillus niger*, and *E. coli* and *S. aureus* by oregano EO, respectively, and found similar results of this study.

Combination of essential oils

The lowest effective volume of 3µL was used for evaluation of the EO blends. Table 4 shows the effects of the blends on microbial growth. The blend cinnamon + sweet fennel was the lowest effective against all microorganisms tested, while the other blends were effective against *E. coli, S. aureus,* and *Penicillium* spp, with similar results among them. The blends containing sweet fennel EO + oregano EO, and sweet fennel + oregano + cinnamon EOs

Table 4. Effect of the essential oils blends (1:1 or 1:1:1) on the microbial growth. Mean inhibition zone diameters (cm) for the antimicrobial activity of 3 µL EO blends against *E. coli, S. aureus* and *Penicilium* spp. Disc diameter is not included.

Blends	Microorganism		
	Escherichia coli	*Staphylococcus aureus*	*Penicillium* spp.
Oregano + Cinnamon	0.54[a]	1.53[a]	2.63[ab]
Oregano + sweet fennel	0.50[a]	3.67[c]	0.87[b]
Oregano + Cinnamon + sweet fennel	0.87[a]	2.15[ac]	3.41[a]
Cinnamon +sweet fennel	0.00[b]	0.00[b]	0.44[c]

*Means within columns followed by the same superscript lower case letter are not significantly different from each other (p> 0.05)

exhibited an improved effect against *E. col*i, while the blend containing sweet fennel EO + oregano EO showed lower effect against *Penicillium* spp.

The positive and negative interactions of EO may depend on both the microorganisms studied and the ratio of the blend components (Harris *et al.* 2002; Radulović *et al.* 2007; Bassolé and Juliani, 2012). When analyzing the effects of EOs, either alone (Table 3) or in combination (Table 4), on the microbial growth, although the best results were found for oregano EO against all microorganisms, lower antimicrobial effects were observed for the blends containing this essential oil, i.e an antagonistic effect may occur when oregano EO is combined with sweet fennel EO and/or cinnamon EO.

The cinnamon EO exhibited less or no effect against the microorganisms studied when combined with sweet fennel EO, probably due to an antagonistic effect of these essential oils against *S. aureus* and *Penicillium*.spp. An antagonistic effect occurs when the combined effect of two or more components is lower than their individual effects (Goñi *et al*., 2009), while a synergistic effect increases the action spectrum, thus improving the effectiveness of the essential oils (Goñi *et al*.,2009). No synergistic effect was observed between the combinations of EOs in the present study

Conclusion

Oregano essential oil was the most effective antimicrobial agent against bacteria and fungi, followed by cinnamon EO and sweet fennel EO. The essential oils in combination showed lower antimicrobial effect when compared to the oregano EO alone. t was observed that the dosage of 3 µL of essential oil was sufficient for inhibition of the microorganisms studied. Essential oils can be used as a substitute for synthetic chemicals in food preservation. An effective combination of essential oils against target microorganisms can lead to a reduction of the pronounced aroma of EO, without affecting the quality of the products.

Acknowledgements

This study was supported by Coordination for the Improvement of Higher Education Personnel (CAPES), Foundation for Research Support in the State of Rio de Janeiro (FAPERJ), National Council for Scientific and Technological Development (CNPq), Dean of Research, Graduate Studies and Innovation / Fluminense Federal University (PROPPI/UFF), Innovation Agency- AGIR / UFF; Rural Federal University of Rio de Janeiro (UFRRJ), and Fluminense Federal University (UFF).

References

Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. 2008. Biological effects of essential oils-A review. Food and Chemical Toxicology 46: 446–475.

Bassolé, I. H. N. and Julian, H. R. 2012. Essential oils in combination and their antimicrobial properties. Molecules 17: 3989-4006.

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods-a review. International Journal of Food Microbiology 94:223-253.

Clinical and Laboratory Standards Institute (CLSI). 2003. Performance standards for antimicrobial disk susceptibility Tests. 8th ed. Approved Standard M2-A8. USA: National Committee for Clinical Laboratory Standards (NCCLS).

Dussault, D., Vu K.D. and Lacroix, M. 2014. In vitro evaluation of antimicrobial activities of various commercial essential oils, oleoresin and pure compounds against food pathogens and application in ham. Meat Science 96: 514-520.

Dvaranauskaite, A., Venskutonis, P., Raynaud, C., Talou, T., Viskelis, P. and Sasnauskas, A. 2009.Variations in the essential oil composition in buds of six

black currant (*Ribes nigrum* L.) cultivars at various development phases. Food Chemistry 114: 671–679.

Food And Drug Administration (FDA). (2015). Code of Federal Regulations (CFR). Title 21: Food and Drugs. Chapter I – Food and Drug Administration, Department of Health and Human Services, Subchapter B – Food for Human Consumption (Continued), Part 182 – Substances Generally Recognized as Safe (GRAS), Subpart A – General Provisions, Subpart 182.20 – Essential oils, oleoresins, and natural extractives. Office of the Federal Register, Washington

Goñi, P., López, P., Sánchez, C., Gómez-Lus, R., Becerril, R. and Nerín, C. 2009. Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. Food Chemistry 116: 982-989.

Harris, R. 2002. Synergism in the essential oil world. The International Journal of Aromatherapy 12: 179-186.

Hill, L. E., Gomes, C. and Taylor, T. M. 2013. Characterization of beta-cyclodextrin inclusion complexes containing essential oils (trans -cinnamaldehyde, eugenol, cinnamon bark, and clove bud extracts) for antimicrobial delivery applications. LWT- Food Science and Technology 51: 86–93.

López, P., Sánchez, C., Batlle,R. and Nerín, C. 2007. Vapor-Phase Activities of Cinnamon, Thyme, and Oregano Essential Oils and Key Constituents against Foodborne Microorganisms. Journal of Agricultural and Food Chemistry 55: 4348−4356.

Lv, J., Huang, H., Yu, L., Whent, M., Niu, Y., Shi, H., Wang, T. T. Y., Luthria, D., Charles, D. and Yu, L. L. 2012. Phenolic composition and nutraceutical properties of organic and conventional cinnamon and peppermint. Food Chemistry 132: 1442–1450.

Nielsen, P.V. and Rios, R. 2000. Inhibition of fungal growth on Bread by volatile components from spices and herbs, an the possible application in active packaging, whit spices emphasis an mustard essential oil. Internacional Journal of Food Microbiology 60: 219-229.

Radulović, N., Mišić, M., Aleksić, J., Đoković, D., Palić, R. and Stojanović, G. 2007. Antimicrobial synergism and antagonism of salicylaldehyde in Filipendula vulgaris essential oil. Fitoterapia 78: 565–570.

Rodríguez, A., Batlle, R. and Nerín, C. 2007. The use of natural essential oils as antimicrobial solutions in paper packaging. Part II. Progress in Organic Coatings 60: 33-38.

Sleha, R., Mosio, P., Vydrzalova, M., Jantovska, A., Bostikova, V. and Mazurova, J. In vitro antimicrobial activities of cinnamon bark oil, anethole, carvacrol, eugenol and guaiazulene against *Mycoplasma hominis* clinical isolates. 2014. Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia Rebublic 158: 208-211.

Sousa, L. L., de Andrade, S. C. A., Athayde, A. J. A. A., de Oliveira, C. E. V., De Sales, C. V., Madruga, M. S. and De Souza, E. L. 2013. Efficacy of *Origanum vulgare* L. and *Rosmarinus officinalis* L. essential oils in combination to control postharvest pathogenic *Aspergillus* and autochthonous mycoflora in *Vitislabrusca* L. (table grapes). International Journal of Food Microbiology 165: 312–318.

Szczepanski, S. and Lipski, A. 2014. Essential oils show specific inhibiting effects on bacterial biofilm formation. Food Control 36: 224-229.

Teixeira, B., Marques, A., Ramos, C., Neng, N. R., Nogueira, J. M. F., Saraiva, J. A. and Nunes, M. L. 2013. Chemical composition and antibacterial and antioxidant properties of commercial essential oils. Industrial Crops and Products 43: 587–595.

Viuda-Martos, M., Mohamady, M. A., Fernández-López, J., A. B. D., Elrazik, K. A., Omer, E. A., Pérez-Alvarez, J. A. and Sendra, E. 2012. *In vitro* antioxidant and antibacterial activities of essentials oils obtained from Egyptian aromatic plants. Food Control 22: 1715-1722.

Wannes, W. A., Mhamdi, B., Sriti, J., Ben Jemia, M., Ouchikh, O., Hamdaoui, G. and Marzouk, B. 2010. Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtuscommunis* var. *italica* L.) leaf, stem and flower. Food and Chemical Toxicology 48: 1362–70.

Wieczynska, J., Luca, A., Kidmose, U., Cavoski, I. and Edelenbos, M. 2016. The use of antimicrobial sachets in the packaging of organic wild rocket: Impact on microorganisms and sensory quality. Postharvest Biology and Technology, 121: 126–134.

Xing, Y., Li, X., Xu, Q., Yun, J. and Lu, Y. 2010. Antifungal activities of cinnamon oil against *Rhizopus nigricans, Aspergillus flavus* and *Penicillium expansum in vitro* and in vivo fruit test. International Journal of Food Science and Technology 45: 1837–1842.

Zinoviadou, K. G., Koutsoumanis, K. P. and Biliaderis, C. G. 2009. Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. Meat Science 82: 338-345.