Journal of Food Chemistry & Nanotechnology

http://dx.doi.org/10.17756/jfcn.2016-020

Research Article

Open Access

Individual and Combined Antimicrobial Activity of Oleuropein and Chemical Sanitizers

L. C. C. Dominciano, C. A. F. Oliveira, S. H. Lee and C. H. Corassin*

Department of Food Engineering, College of Animal Science and Food Engineering, University of São Paulo, Av. Duque de Caxias Norte, 225, CEP 13635-900 - Pirassununga, SP, Brazil

***Correspondence to:**
C. H. Corassin
Department of Food Engineering
College of Animal Science and Food Engineering, University of São Paulo
Av. Duque de Caxias Norte, 225
CEP 13635-900 - Pirassununga, SP, Brazil
E-mail: carloscorassin@usp.br

Received: May 09, 2016
Accepted: August 03, 2016
Published: August 05, 2016

Citation: Dominciano LCC, Oliveira CAF, Lee SH, Corassin CH. 2016. Individual and Combined Antimicrobial Activity of Oleuropein and Chemical Sanitizers. *J Food Chem Nanotechnol* 2(3): 124-127.

Published by United Scientific Group

Abstract

In this study, the minimum inhibitory concentration (MIC) of oleuropein (OLE), a phenolic compound extracted from Oliveira leaves, alone or in combination with commercial sanitizers, was determined for *Listeria monocytogenes* (ATCC 7644), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). MIC values were determined using individual suspensions containing nearly 10^8 cells/mL of each bacterial species treated with serial dilutions of OLE (0.4 mg/mL) and commercially available solutions of Peracetic acid (PAA, 2.0%), Benzalkonium chloride (BC, 1.0%), Chlorhexidine digluconate (CD, 2.0%), Sodium hypochlorite (SH, 2.0%) and Hydrogen peroxide (HP, 3.0%). OLE had low inhibitory activity (0.2 mg/mL). As expected commercial sanitizers showed high bactericidal activity according to MIC values. However, the association of OLE with commercial sanitizers increased their bactericidal effect, especially for CD, which combination with OLE resulted in approximately 60-fold reduction in the MIC values for *S. aureus* and *L. monocytogenes*. OLE and BC also led to nearly 30-fold or 10-fold reductions of MIC values for *S. aureus* and *L. monocytogenes*, respectively. The combination of PAA and OLE reduced the MIC values for *E. coli* and *S. aureus* by 10-fold, with little effect on *L. monocytogenes*. Results of this preliminary study indicate that OLE has the potential to enhance the bactericidal effect of commercial sanitizers, especially against *L. monocytogenes* and *S. aureus*. Further studies are necessary to understand the mechanisms of action of these combinations.

Keywords

Phenolic compounds, Sanitizers, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*

Introduction

Listeria monocytogenes, *Staphylococcus aureus* and *Escherichia coli* are among the most important foodborne pathogens, being also able to form biofilms and persist in the food industry environment [1]. The bacterial load of equipment and utensils can be effectively reduced by adequate cleaning and disinfection procedures [2]. However, the effectiveness of a particular sanitizer can be affected by several factors, including its concentration, contact time at a given temperature, pH, interaction with inactivating substances or type of bacteria, Gram-negative or Gram-positive [3]. Sanitizers should have low toxicity, be stable in variable condition of use, have a broad spectrum of antimicrobial activity and quickly destroy the agents, but there is still no single product with all these features [4]. Peracetic acid (PAA), Benzalkonium chloride (BC), Chlorhexidine digluconate (CD), Sodium hypochlorite (SH) and Hydrogen peroxide (HP) are active compounds of many

commercial sanitizers used for disinfection of equipment and utensils in the food industry. PAA has a broad antimicrobial spectrum and is a strong oxidizing agent that is decomposed into safe waste products. However, PAA is highly corrosive and may be of difficult handling and storage [5]. BC has low toxicity profile and is stable in work solutions, but it leaves residues on surfaces after rinse [6]. CD also has a broad spectrum, despite the advantages of being pH-dependent and having reduced activity in the presence of organic matter [7]. SH is a low cost sanitizer with broad spectrum activity, although it is unstable, corrosive and pH-dependent [8]. HP is a widely used biocide for disinfection, sterilization, and antisepsis, being environmentally friendly because it can rapidly degrade into water and oxygen. However, HP can be corrosive to metal surfaces and may cause chemical burns or explosions at high concentrations [5].

The individual action of chemical sanitizers may be enhanced by their association with other compounds, such as humectants, dispersants and chelates, in the formulations of commercial sanitizers. Moreover, high concentrations of a sanitizer may be effective to inactivate susceptible bacterial cells, although some cells may undergo natural resistance through mutation or genetic exchange [1]. This resistance can jeopardize the sanitizer's effectiveness, which poses a serious risk of contamination of food processing lines and final products [9].

Oleuropein (OLE), a heterocyclic ester, glycoside secoiridoide is the most abundant phenolic compound found in olive tree, *Olea europaea* [10]. OLE plays an important role in the plant's defense against microbial pathogens and insects [11]. Although OLE is not classified as a sanitizer, previous studies have indicated antimicrobial activity of OLE on *L. monocytogenes*, *S. aureus* and several species of enterobacteria, among others [12, 13]. The minimum inhibitory concentration (MIC) values reported for OLE on *S. aureus* varies from 0.0625 to 0.5 mg/mL for certified strains of the American Type Culture Collection (ATCC), and from 0.0312 to 0.250 mg/mL for clinical isolates [14]. OLE solution at 0.4 mg/mL completely inhibited the growth of *E. coli*, *Klebsiella pneumoniae* and *Bacillus cereus* [15]. However, there is no previous study on the antimicrobial activity of the combination of OLE and chemical sanitizers against bacterial cells. Therefore, the objective of the present study was to determine the MIC values of OLE and chemical sanitizers commonly used in the food industry, alone or in combination, on suspended cells of *S. aureus*, *E. coli* and *L. monocytogenes*.

Material and Methods

Bacterial isolates

Commercially available strains of *S. aureus* (ATCC 25.923), *L. monocytogenes* (ATCC 7.644) and *E. coli* (ATCC 25.922) (CEFAR®, Brazil) were used in the study. Each strain was previously suspended in Brain Heart Infusion broth (BHI) (Merck, Germany) with glycerol (Synth, Brazil) 15%, and stored at -80 °C. Each bacterial working suspension was prepared by adding 15 µL of the stock suspension to 5 mL of BHI. The tubes were incubated at 30 °C for 24 h, vortexed and finally adjusted with BHI until reaching 0.5 in the MacFarland scale (approximately 10^8 cells/mL).

Oleuropein and commercial sanitizers

OLE (purity: >80%) was purchased from Sigma-Aldrich (Saint Louis, MO) and diluted in 100 mL sterilized water, to prepare a working solution containing 0.4 mg/mL. The following chemical sanitizers were purchased (Dinâmica, São Paulo) and diluted in duplicate at concentrations recommended by the manufacturer: Peracetic acid (2.0%), Benzalkonium chloride (1.0%), Chlorhexidine digluconate (2.0%), Sodium hypochlorite (2.0%) and Hydrogen peroxide (3.0%). One replicate set of sanitizers solutions were used for preparing working solutions of combination of the sanitizers at the same concentrations described with OLE (0.4 mg/mL).

Determination of minimum inhibitory concentration (MIC)

MIC values were determined for OLE and each chemical sanitizer, as well as for their combinations with OLE, according to the procedures as described by the National Committee for Clinical Laboratory Standards [16], as follows. Twelve tubes were prepared with culture medium (BHI for *S. aureus* and *E. coli*, and BHI supplemented with yeast extract at 0.6% for *L. monocytogenes*), to which 10 mL of serial dilutions (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 and 1:512) of each sanitizing compound were added. Additional 12 tubes were prepared with the same dilutions for each of the combinations of OLE and the sanitizers. The tubes were inoculated with 100 µL of each bacterial suspension. Additional tubes were prepared with the sanitizers and BHI broth (negative control), and with the bacterial suspensions and BHI broth (positive control). The tubes were incubated at 37 °C for 24 h, and subjected to visual inspection. The assays were prepared in triplicate, and individual MIC values were determined as the lowest concentration of the sanitizing agent that prevented visible growth (without turbidity) of the microorganism [16]. Final results were expressed as mean MIC values (n=3).

Results and Discussions

The results of MIC for OLE and the five chemical sanitizers tested are presented in Table 1. The MIC value for OLE was 0.2 mg/mL, for the three bacterial species tested. The isolates showed different responses to the sanitizers, as *E. coli* had higher MIC for BC (0.156 mg/mL) followed by HP (0.234 mg/mL), PAA and CD (0.312 mg/mL); *S. aureus* had less resistance to HP (MIC: 0.117 mg/mL), SH and PAA (MIC: 0.312 mg/mL). *L. monocytogenes* showed the lowest resistance to PAA (MIC: 0.156 mg/mL), HP (MIC: 0.468 mg/mL) and BC (MIC: 0.625 mg/mL).

Figure 1 presents the ratios for MIC values for the sanitizers evaluated alone and their combinations with OLE against suspended cells of *L. monocytogenes*, *S. aureus* and *E. coli*. Compared to CD alone, its combination with OLE resulted in approximately 60-fold reduction in the MIC values for *S. aureus* and *L. monocytogenes*. However, the bactericidal effect of OLE and the sanitizers tested was not fully determined in the present study since no enumeration of initial and final bacterial population was done.

Table 1: Minimum inhibitory concentration values for oleuropein and commercial sanitizers, alone or in combination, against suspended cells of *Listeria monocytogenes* (ATCC 7644), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922).

	Minimum inhibitory concentration (mg/mL)		
Compound or mixture	***E. coli***	***S. aureus***	***L. monocytogenes***
OLE	0.200	0.200	0.200
PAA	0.312	0.312	0.156
PAA + OLE	< 0.039*	< 0.039*	< 0.039*
BC	0.156	1.250	0.625
BC + OLE	0.039	0.039	0.078
CD	0.312	2.500	2.500
CD + OLE	0.078	< 0.039*	< 0.039*
SH	2.500	0.312	1.250
SH + OLE	0.625	0.156	0.312
HP	0.234	0.117	0.469
HP + OLE	0.234	< 0.058*	0.234

OLE: Oleuropein; PAA: Peracetic acid; BC: Benzalkonium chloride; CD: Chlorhexidine digluconate; SH: Sodium hypochlorite; HP: Hydrogen peroxide.

*Tubes with no sign of turbidity after incubation at 37 °C, 24 hours.

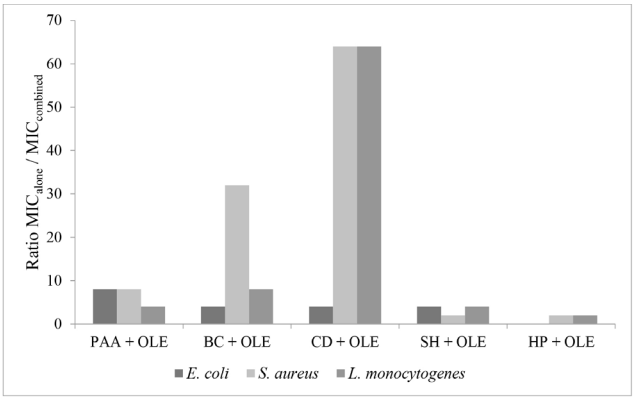

Figure 1: Ratios for minimum inhibitory concentrations (MIC) of sanitizers evaluated alone and their combinations with oleuropein (OLE) against suspended cells of Listeria monocytogenes (ATCC 7644), Staphylococcus aureus (ATCC 25923) and *Escherichia coli* (ATCC 25922). PAA: Peracetic acid; BC: Benzalkonium chloride; CD: Chlorhexidine digluconate; SH: Sodium hypochlorite; HP: Hydrogen peroxide.

OLE and BC also led to nearly 30-fold or 10-fold reductions of MIC values for *S. aureus* and *L. monocytogenes*, respectively. The combination of PAA and OLE reduced the MIC values for *E. coli* and *S. aureus* by 10-fold, with little effect on *L. monocytogenes*.

The MIC value for OLE (0.2 mg/mL) is similar to those reported by Bisignano et al. [14], who obtained antibacterial effects in OLE at concentrations ranging from 0.03 to 0.2 mg/mL on 42 Gram-negative and Gram-positive bacterial isolates, including *S. aureus* (ATCC 25.923). The inhibitory effects of OLE may increase at higher concentrations of the compound [2]. OLE solution at 0.5 mg/mL against 15 bacterial species, including *E. coli* and *S. aureus*, was able to inhibit all species tested, except for *S. aureus* and *Salmonella enteritidis* [8]. These differences may be partially explained by the differences in the strains used and the composition of the extract containing OLE, because the extraction processes uses organic solvents, which can modify the polarity of the compound [2]. The mechanisms of antimicrobial activity of OLE are not completely understood. OLE and other compounds in olive oil interact with phosphatidylglycerol at the surface of the bacterial cell wall, and cause changes in the cytoplasmic membrane which could lead to the disruption of the cell envelope [12, 13].

By definition, a sanitizer is a type of antimicrobial that (according to EPA specifications) kills or irreversibly inactivates at least 99.9% (3-log reduction) of all bacteria. In this study, PAA, BC, CD, SH and HP were considered effective against the bacterial species tested. However, the presence of organic matter can reduce the disinfection effect of the sanitizer [5]. Thus, the routine hygiene practices in food industries should be remove efficiently organic wastes to allow the complete action of the sanitizer to prevent food contamination, total losses of food batches and interruption of production processes [3].

When the chemical sanitizers tested were mixed to OLE, they resulted in greater efficiency against the microorganisms, as presented in Table 1. There are no previous studies with the association of OLE and chemical sanitizers, except for a report by [11], who also observed a cooperative effect between at higher levels of OLE (5%) and HP (5%) against *S. aureus*. The antimicrobial effect of CD and OLE was higher than its combinations with other sanitizers, which resulted in approximately 60-fold reduction in the MIC values for *S. aureus* and *L. monocytogenes*. This result indicates a synergist effect between the compounds, and suggests a possible interaction of OLE with the chlorhexidine cationic molecule. Besides having a wide range of antimicrobial activity, its cationic structure provides a unique property named substantively, which allows its continuing action even after removal [7]. In high dosages, CD causes precipitation and coagulation of proteins and bacterial cytoplasmic death, while at lower dose, the integrity of cell membrane is altered, resulting in overflow of bacterial components of low molecular weight [17]. However, it remains to be determined if reductions in MIC values observed in this study may have occurred as a result of molecular interactions between OLE and CD.

Although the mechanisms of action of BC on the microbial cell remains unclear, it is widely accepted that it targets cytoplasmic membrane, causing structural loss in its organization and integrity, along with the extravasation of cell components due to disruption of the membrane, as well as protein denaturation and enzymes [6]. PAA is a potent sanitizer [5], being active in low concentration against a wide spectrum of organisms while others sanitizers (e.g. HP) require doses much higher that PAA for the same level of disinfection. PAA interferes with the selective function of the cytoplasmic membrane and ruptures the walls of the Gram-positive cells, and is also equally effective against the membrane lipoproteins, facilitating their action against Gram-negative microorganism

[4]. Therefore, the combinations of OLE and BC or PAA probably resulted in an additive effect, taking into account that they resulted in up to 30-fold reductions of MIC values for the bacterial species studied.

Conclusions

OLE had low inhibitory activity against bacterial suspensions of certified strains of *S. aureus*, *L. monocytogenes* and *E. coli*. However, the association of OLE with commercial sanitizers increased their bactericidal effect. These preliminary results indicate that OLE has the potential to enhance the bactericidal effect of commercial sanitizers, especially against *L. monocytogenes* and *S. aureus*. Further studies are necessary to better understand the mechanisms of action of these combinations.

References

1. Simões M, Simões LC, Vieira MJ. 2010. A review of current and emergent biofilm control strategies. *LWT-Food Sci Technol* 43(4): 573-583. doi: 10.1016/j.lwt.2009.12.008
2. Moretro T, Heir E, Nesse LL, Vestby LK, Langsrud S. 2012. Control of *Salmonella* in food related environments by chemical disinfection. *Food Res Int* 45(2): 532-544. doi: 10.1016/j.foodres.2011.02.002
3. Kitis M. 2004. Disinfection of wastewater with peracetic acid: a review. *Environ Int* 30(1): 47-55. doi: 10.1016/S0160-4120(03)00147-8
4. Beraldo C, Daneluzzi NS, Scanavacca J, Doyamam JT, Fernandes AJ, et al. 2013. Eficiência de óleos essenciais de canela e cravo-da-índia como sanitizantes na indústria de alimentos. *Pesquisa Agropecuária Tropical* 43(4): 436-440.
5. Wagner M, Brumelis D, Gehr R. 2002. Disinfection of wastewater by hydrogen peroxide or peracetic acid: development of procedures for measurement of residual disinfectant and application to a physicochemically treated municipal effluent. *Water Environ Res* 74(1): 33-50. doi: 10.2175/106143002X139730
6. Merianos JJ. 1991. Quaternary ammonium compounds. In: Block SS (eds) Disinfection, sterilization, and preservation. Lea and Febiger, Philadelphia, USA, pp 225-255.
7. Mohammadi Z, Abbott PV. 2009. The properties and applications of chlorhexidine in endodontics. *Int Endod J* 42(4): 288-302. doi: 10.1111/j.1365-2591.2008.01540.x
8. Riazi S, Matthews KR. 2011. Failure of foodborne pathogens to develop resistance to sanitizers following repeated exposure to common sanitizers. *Int Biodeterior Biodegradation* 65(2): 374-378. doi: 10.1016/j.ibiod.2010.12.001
9. Stijn VDV, Abee T. 2011. Mixed species biofilms of *Listeria monocytogenes* and *Lactobacillus plantarum* show enhanced resistance to benzalkonium chloride and peracetic acid. *Int J Food Microbiol* 144(3): 421-431. doi: 10.1016/j.ijfoodmicro.2010.10.029
10. Benavente-Garcia O, Castillo J, Lorente J, Ortuño A, Del Rio JA. 2000. Antioxidant activity of phenolics extracted from *Olea europaea* leaves. *Food Chem* 68(4): 457-462. doi: 10.1016/S0308-8146(99)00221-6
11. Zanichelli D, Baker TA, Clifford MN, Adams MR. 2005. Inhibition of *Staphylococcus aureus* by oleuropein is mediated by hydrogen peroxide. *J Food Prot* 68(7): 1492-1496.
12. Medina E, de Castro A, Roero C, Brenes M, 2006. Comparison of the concentrations of phenolic compounds in olive oils and other plant oils: correlation with antimicrobial activity. *J Agric Food Chem* 54(14): 4954-4961. doi: 10.1021/jf0602267
13. Laincer F, Laribi R, Tamendjari A, Arrar L, Rovellini P, et al. 2014. Olive oils from Algeria: phenolic compounds, antioxidant and antibacterial activities. *Grasas y Aceites* 65(1): e001. doi: 10.3989/gya.035713
14. Bisignano G, Tomaino A, Cascio RL, Crisafi G, Uccella N, et al. 1999. On the *in vitro* antimicrobial activity of oleuropein and hydroxytyrosol. *J Pharm Pharmacol* 51(8): 971-974. doi: 10.1211/0022357991773258
15. Aziz NH, Farag SE, Mousa LA, Abo-Zaid MA. 1998. Comparative antibacterial and antifungal effects of some phenolic compounds. *Microbios* 93(374): 43-54.
16. National Committee for Clinical Laboratory Standards. 2003. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically; approved standard (6th ed). NCCLS Document M7-A6. Wayne 23, USA.
17. Rölla G, Melsen B. 1975. On the mechanism of the plaque inhibition by chlorhexidine. *J Dent Res* 54: 57-62. doi: 10.1177/00220345750540022601