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Original article

Antibacterial effects of hydrogen peroxide and caprylic acid on selected foodborne bacteria

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Abstract

Bactericidal activity of caprylic acid (CA) and hydrogen peroxide (HP) was investigated in this study in order to design a suitable formulation for use in the food-processing industry. Antibacterial effects of the two chemicals were tested *in vitro* against the reference strains of *Salmonella enterica* subsp. *enterica* serotype Enteritidis CCM 4420, *Escherichia coli* CCM 3988, *Listeria monocytogenes* CCM 5578 and *Staphylococcus aureus* CCM 4223, as well as against the wild bacterial strains obtained from various food commodities (poultry meat, rabbit meat, raw milk sheep cheese 'Bryndza') and potable water. First, suspension test was carried out to determine the minimum bactericidal concentrations for individual chemical compounds. While most Gram-negative bacteria tested were effectively inhibited by HP at a 0.5% concentration, the growth of Gram-positive bacterial strains was stopped by a 2% solution. CA showed similar antibacterial effect on all bacterial strains tested except for *Staph. aureus* showing the same susceptibility as Gram-negative bacteria. The wild strains generally had higher resistance to both chemicals than the reference strains. Combination of HP and CA at concentrations of 0.01%; 0.05% and 0.1% was further tested by the suspension test, carrier test, and carrier test with simultaneous exposure to UV light. The total bactericidal activity against selected foodborne pathogens was already observed at a concentration of 0.1% and the efficiency was significantly increased by the use of UV radiation. A novel disinfectant based on the combination of HP with CA appears to be a suitable binary formulation for potential use in the food sector.

Key words: antibacterial activity, caprylic acid, hydrogen peroxide, *Listeria monocytogenes Salmonella*

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Introduction

Currently, there is a strong competition on the global food market. To succeed, the primary requirement for food business operators is the production of high-quality foods that do not pose any health risk for the consumers. New legislative provisions stipulate strict criteria which are based on professional expertise in this field and must be followed by all food producers. The risk of food contamination can be reduced by effective sanitation procedures including cleaning, disinfection, insect and rodent controls (Laktičová et al. 2005, 2006).

Food plays an important role in the transmission of both food-borne and food-related diseases. Therefore, the level of hygiene throughout the food sector is one of the key factors ensuring production of high quality and safe products. With regard to a permanent increase in resistance of microorganisms to chemical compounds used in the environment, primary agricultural production and food-processing establishments, there is a need to develop novel sanitizing agents based on a combination of several chemical compounds (multi-component disinfectants). The use of such formulations is simplified by combining suitable components that show both cleaning and disinfecting effects (Štefkovičová et al. 2007). A combination of two or more components may provide a better effect than the sum of the effects of each single compound at the same doses (Wang et al. 2012). When designing combinations of active ingredients, certain rules are to be followed in order to reduce or suppress negative effects of one or all the components in the formulation resulting from their reactivity and solubility as well as changes in pH values (Štefkovičová et al. 2007).

This study was directed to design a novel two-component disinfectant suitable for use in food industry with the possibly best bactericidal activity against selected Gram-negative and Gram-positive foodborne bacteria.

Materials and Methods

Test microorganisms

Overnight *BHI* broth (Oxoid, UK) cultures of four reference strains (CCM) and seven wild strains (WS) isolated from food samples were used for testing bactericidal effect of hydrogen peroxide (HP) and caprylic acid (CA). Reference strains obtained from the Czech Collection of Microorganisms in Brno (Czech Republic) included *Salmonella enterica* subsp. *enterica* serotype Enteritidis CCM 4420, *Escherichia coli* CCM 3988, *Listeria monocytogenes* CCM 5578 and *Staphylococcus aureus* CCM 4223. The following wild strains of foodborne bacteria were tested in this study: *Salmonella* Enteritidis and *Escherichia coli* (origin – poultry meat), *Listeria monocytogenes* (origin – raw milk sheep cheese 'Bryndza'), *Staphylococcus aureus* (origin – potable water, 'Bryndza', poultry meat and rabbit meat). The identity of bacterial strains isolated from food samples was confirmed by the species-specific PCR methods for *Salmonella* Enteritidis (Wang and Yeh 2002), *E. coli* (Wang et al. 1996) and *Staphylococcus aureus* (Stromenger et al. 2003). Both the *Listeria monocytogenes* strain isolated from 'Bryndza' as well as the *Staphylococcus aureus* strain isolated from potable water were kindly provided by the Veterinary and Food Institute in Dolný Kubín (Slovakia).

Suspension test

Susceptibility of selected bacterial strains to hydrogen peroxide (VULM SK, Slovakia) and caprylic acid (Sigma Aldrich, USA), both individual and in a combination, was tested by the suspension test according to STN EN 1650 (2020). Bacterial suspensions with an optical density of 10^8 CFU/mL (equal to 0.5 McFarland turbidity standard) were further diluted in a sterile saline solution and the 10^{-5} decimal dilution was used for testing. First, the negative control was prepared by mixing 0.1 mL of the respective diluted bacterial culture with 9.9 mL saline. In the same way, the 0.1 mL aliquots of bacterial cultures were transferred into series of test tubes with 9.9 mL of individual chemicals or a combination thereof.

The following concentrations of HP and CA were used: 0.01%, 0.05%; 0.1%, 0.5%, 1%, 2% and 3% for *S. aureus;* 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1% and 2% for *S.* Enteritidis, *E. coli* and *L. monocytogenes;* 0.01%, 0.05% and 0.1% for a combination of both chemical agents tested. After the exposure for 15 minutes, 0.1 mL aliquots of bacterial suspensions were spread on the surface of the selective culture media (Oxoid, UK) – Baird Parker agar was used for *Staph. aureus*; Endo agar for *S.* Enteritidis and *E. coli*; Columbia blood agar for *L. monocytogenes*. The inoculated plates were incubated for 24 h at 37°C and the results were expressed as the counts of colony forming units (CFU) per 1 mL. To allow reliable statistical evaluation, all the tests were carried out in six replicates. Plates without the addition of any chemical agent were simultaneously inoculated, incubated and used as a negative control.

Table 1. Antibacterial effect of hydrogen peroxide on the reference (CCM) and wild strains (WS) of selected foodborne bacteria.

Concentration	*Salmonella* Enteritidis log CFU/mL		*Escherichia coli* log CFU/mL		*Listeria monocytogenes* log CFU/mL	
	CCM 4420	WS (poultry)	CCM 3988	WS (poultry)	CCM 5578	WS ('Bryndza')*
	$c_0 = 8.18^{Ca}$	$c_0 = 8.18^{Ca}$	$c_0 = 7.97^{Da}$	$c_0 = 7.97^{Da}$	$c_0 = 8.32^{Aa}$	$c_0 = 8.26^{Ba}$
0.005%	8.18^{Ca}	8.18^{Ca}	7.10^{Da}	7.97^{Dab}	8.31^{Aa}	8.25^{Ba}
0.01%	8.17^{Ba}	8.18^{Ba}	7.48^{Db}	7.96^{Cb}	8.25^{Ab}	7.97^{Cb}
0.05%	8.12^{Cb}	8.17^{Aa}	0^{Ec}	6.74^{Ec}	8.15^{Bc}	7.91^{Dc}
0.1%	0^{Cc}	6.72^{Cb}	0^{Cc}	0^{Cd}	8.04^{Ad}	7.11^{Bd}
0.5%	0^{Cc}	0^{Cc}	0^{Cc}	0^{Cd}	7.18^{Ae}	7.08^{Bd}
1.00%	0^{Bc}	0^{Bc}	0^{Bc}	0^{Bd}	7.04^{Ae}	7.04^{Ad}
2.00%	0^{Ac}	0^{Ac}	0^{Ac}	0^{Ad}	0^{Af}	0^{Ae}

* 'Bryndza' – raw milk sheep cheese; CCM – Czech Collection of Microorganisms (Brno, Czech Republic); c_0 – initial concentration; $^{A-E}$ – mean values with different superscript letters in the same row differ significantly ($p<0.05$); $^{a-f}$ – mean values with different superscript letters in the same column differ significantly ($p<0.05$).

Table 2. Antibacterial effect of caprylic acid on the reference (CCM) and wild strains (WS) of selected foodborne bacteria.

Concentration	*Salmonella* Enteritidis log CFU/mL		*Escherichia coli* log CFU/mL		*Listeria monocytogenes* log CFU/mL	
	CCM 4420	WS (poultry)	CCM 3988	WS (poultry)	CCM 5578	WS ('Bryndza')*
	$c_0 = 8.18^{Ca}$	$c_0 = 8.18^{Ca}$	$c_0 = 7.97^{Da}$	$c_0 = 7.97^{Da}$	$c_0 = 8.32^{Aa}$	$c_0 = 8.26^{Ba}$
0.005%	8.18^{Ca}	8.18^{Ca}	7.96^{Da}	7.97^{Da}	8.32^{Aa}	8.25^{Ba}
0.01%	8.18^{Ca}	8.18^{Ca}	7.95^{Da}	7.97^{Da}	8.30^{Ab}	8.23^{Bb}
0.05%	8.17^{Ca}	8.17^{Ca}	7.94^{Da}	7.97^{Dab}	8.26^{Ac}	8.19^{Bc}
0.1%	0^{Eb}	8.17^{Ba}	7.04^{Db}	7.95^{Cb}	8.19^{Ad}	7.95^{Cd}
0.5%	0^{Cb}	0^{Cb}	0^{Cc}	0^{Cc}	7.81^{Ae}	7.59^{Be}
1.00%	0^{Bb}	0^{Bb}	0^{Bc}	0^{Bc}	7.32^{Af}	0^{Bf}
2.00%	0^{Ab}	0^{Ab}	0^{Ac}	0^{Ac}	0^{Ag}	0^{Af}

* 'Bryndza' – raw milk sheep cheese; CCM – Czech Collection of Microorganisms (Brno, Czech Republic); c_0 – initial concentration; $^{A-E}$ – mean values with different superscript letters in the same row differ significantly ($p<0.05$); $^{a-f}$ – mean values with different superscript letters in the same column differ significantly ($p<0.05$).

Carrier test without/with simultaneous exposure to UV light

The test was performed using ceramic tiles of 10 x 10 cm. An 0.1 mL aliquot of bacterial suspension was transferred to a pre-sterilised tile and spread over the entire surface with a sterile glass rod. Then 9.9 mL volumes of the two chemicals tested or the combination thereof were applied to the inoculated tile surface. After the exposure for 15 min without/with simultaneous exposure to UV radiation (Prolux G Nexa, Slovakia) at 260 nm, swabs were taken from the entire tile surface. Each swab was transferred into a tube with 10 mL of sterile saline solution and shaken for 10 min. After that, a volume of 0.1 mL was spread on the surface of a particular culture medium as described above for the suspension test. The inoculated plates were incubated at appropriate temperatures and bacterial growth was compared to negative control plates without chemical agents.

Results

Suspension test

Antibacterial activity of HP and CA against all reference strains as well as strains of *S.* Enteritidis, *E. coli* and *L. monocytogenes* isolated from food samples is presented in Tables 1 and 2. Both chemicals

Table 3. Antibacterial effect of hydrogen peroxide on the reference (CCM) and wild strains (WS) of *Staphylococcus aureus*.

Concentration	*Staphylococcus aureus* log CFU/mL				
	CCM 4223 $c_0 = 8.15^{Ca}$	WS (poultry) $c_0 = 8.23^{Aa}$	WS ('Bryndza')* $c_0 = 8.11^{Da}$	WS (water) $c_0 = 8.18^{Ba}$	WS (rabbit) $c_0 = 8.18^{Ba}$
0.01%	7.65^{Db}	8.20^{Ab}	8.11^{Ba}	7.97^{Cb}	7.99^{Cb}
0.05%	7.53^{Ec}	8.15^{Ac}	8.04^{Bb}	7.74^{Dc}	7.89^{Cc}
0.1%	7.43^{Ed}	7.74^{Cd}	7.99^{Ac}	7.63^{Dd}	7.81^{Bd}
0.5%	7.40^{Ed}	7.70^{Cd}	7.97^{Acd}	7.59^{Dde}	7.77^{Bd}
1.00%	7.26^{Ee}	7.68^{Cd}	7.95^{Ad}	7.57^{Dde}	7.72^{Be}
2.00%	0^{Cf}	0^{Ce}	0^{Ce}	7.54^{Be}	7.62^{Af}
3.00%	0^{Af}	0^{Ae}	0^{Ae}	0^{Af}	0^{Ag}

* 'Bryndza' – raw milk sheep cheese; CCM – Czech Collection of Microorganisms (Brno, Czech Republic); c_0 – initial concentration; $^{A-E}$ – mean values with different superscript letters in the same row differ significantly ($p<0.05$); $^{a-f}$ – mean values with different superscript letters in the same column differ significantly ($p<0.05$).

Table 4. Antibacterial effect of caprylic acid on the reference (CCM) and wild strains (WS) of *Staphylococcus aureus*.

Concentration	*Staphylococcus aureus* log CFU/mL				
	CCM 4223 $c_0 = 8.15^{Ca}$	WS (poultry) $c_0 = 8.23^{Aa}$	WS ('Bryndza')* $c_0 = 8.11^{Da}$	WS (water) $c_0 = 8.18^{Ba}$	WS (rabbit) $c_0 = 8.18^{Ba}$
0.01%	8.14^{Ca}	8.23^{Aa}	8.11^{Da}	8.17^{Ba}	8.18^{Ba}
0.05%	8.11^{Cb}	8.21^{Ab}	8.11^{Ca}	8.16^{Ba}	8.15^{Bb}
0.1%	8.00^{Dc}	8.18^{Ac}	8.10^{Ba}	8.04^{Cb}	7.43^{Ec}
0.5%	0^{Ad}	0^{Ad}	0^{Ab}	0^{Ac}	0^{Ad}

* 'Bryndza' – raw milk sheep cheese; CCM – Czech Collection of Microorganisms (Brno, Czech Republic); c_0 – initial concentration; $^{A-E}$ – mean values with different superscript letters in the same row differ significantly ($p<0.05$); $^{a-f}$ – mean values with different superscript letters in the same column differ significantly ($p<0.05$).

inhibited the reference strain of *S.* Enteritidis at a concentration as low as 0.1%. To achieve the same bactericidal effect on *S.* Enteritidis strain originating from poultry meat, 0.5% concentration was required. However, the exposure of the same strain to 0.1% HP resulted in a significant decrease ($p<0.05$) in bacterial cell counts (from 10^8 to 10^6 CFU/mL).

Antibacterial effect of HP on *E. coli* reference strain was visible better than that on *S.* Enteritidis – the cells were already devitalised at 0.05% concentration. The reference strain was also less resistant to CA; its exposure to 0.1% concentration resulted in a slight reduction of bacterial counts and the 0.5% concentration completely stopped any bacterial growth.

Both tested chemicals devitalised *L. monocytogenes* at 2% concentration. Lower sensitivity of the reference strain was observed to CA. Bactericidal effect of CA on *L. monocytogenes* strain isolated from 'Bryndza' was observed at 1% concentration, while the reference strain was not completely devitalised by this concentration. Increasing concentrations of HP and CA gradually decreased viable bacterial cell counts (Tables 1 and 2).

Antibacterial activity of CA and HP to the reference strain and food originating strains of *Staph. aureus* is shown in Tables 3 and 4. The results confirmed bactericidal effect of CA on the reference strain and all the wild strains of staphylococci at concentrations equal to or higher than 0.5%. Bactericidal effect of HP was confirmed in all strains of *Staph. aureus* at the highest concentration tested (3%); a 2% concentration stopped the growth of the reference strain and strains of *Staph. aureus* isolated from poultry meat and 'Bryndza'. As seen in Table 3, increasing concentrations of HP resulted in a gradual moderate decrease in bacterial counts.

Antibacterial activity of HP and CA in a combination (suspension test, carrier test, carrier test and UV)

Combinations of both HP and CA were prepared according to results of the suspension test with indivi-

Table 5. Antibacterial effects of the combination of hydrogen peroxide and caprylic acid on the reference (CCM) and wild strains (WS) of selected foodborne bacteria.

Bacterial strain	Initial concentration log CFU/mL	Suspension test			Carrier test			Carrier test and UV		
		0.01%	0.05%	0.10%	0.01%	0.05%	0.10%	0.01%	0.05%	0.10%
		log CFU/mL			log CFU/mL			log CFU/mL		
S. Enteritidis										
CCM 4420	7.66^{Ad}	5.96^{Cf}	5.75^{Ce}	0^{Ca}	6.87^{Be}	6.72^{Be}	0^{Ca}	4.11^{Cd}	3.81^{Ce}	0^{Ca}
WS (poultry)	7.54^{Ae}	7.50^{Bc}	6.79^{Cd}	0^{Da}	5.85^{Df}	5.81^{Df}	0^{Da}	5.63^{Dd}	5.50^{Dd}	0^{Da}
E. coli										
CCM 3988	7.27^{Af}	7.07^{Be}	5.99^{De}	0^{Da}	7.00^{Cde}	5.96^{Df}	0^{Da}	5.56^{Dd}	4.90^{De}	0^{Da}
WS (poultry)	7.75^{Ac}	7.39^{Cd}	6.66^{Dd}	0^{Ea}	7.54^{Bc}	6.72^{De}	0^{Ea}	6.81^{Dc}	5.85^{Ec}	0^{Ea}
L. monocytogenes										
CCM 5578	7.92^{Aa}	7.81^{Ca}	7.60^{Da}	0^{Fa}	7.85^{Ba}	7.79^{Ca}	0^{Fa}	6.65^{Ec}	5.51^{EFd}	0^{Fa}
WS ('Bryndza')	7.75^{Ac}	7.70^{Bb}	7.32^{Eb}	0^{Fa}	7.72^{ABb}	7.66^{Cb}	0^{Fa}	7.50^{Db}	6.25^{Fb}	0^{Fa}
Staph. aureus										
CCM 4223	7.87^{Ab}	7.68^{Bb}	7.56^{Ca}	0^{Fa}	7.51^{CDc}	7.25^{Ec}	0^{Fa}	7.46^{Db}	6.25^{Fb}	0^{Fa}
WS (water)	7.74^{Ac}	7.11^{Be}	5.43^{De}	0^{Da}	7.74^{Ab}	6.80^{Ce}	0^{Da}	7.72^{Aa}	6.71^{Ca}	0^{Da}
WS ('Bryndza')*	7.79^{Ac}	6.72^{Bf}	0^{Ce}	0^{Ca}	5.73^{Cf}	0^{Cf}	0^{Ca}	5.14^{Cd}	0^{Ce}	0^{Ca}
WS (rabbit)	7.61^{Ade}	7.17^{Be}	6.85^{Cd}	0^{Da}	7.17^{Bd}	6.90^{Cde}	0^{Da}	6.88^{Cc}	5.86^{Dc}	0^{Da}
WS (poultry)	7.63^{Ad}	7.34^{Bd}	7.14^{Cc}	0^{Ea}	7.04^{CDde}	7.0^{Dd}	0^{Ea}	5.94^{Ed}	5.90^{Ec}	0^{Ea}

* 'Bryndza' – raw milk sheep cheese; CCM – Czech Collection of Microorganisms (Brno, Czech Republic); $^{A-E}$ – mean values with different superscript letters in the same row differ significantly ($p<0.05$); $^{a-f}$ – mean values with different superscript letters in the same column differ significantly ($p<0.05$).

dual chemicals. Antibacterial activity of HP and CA in a combination at concentrations of 0.01%, 0.05% and 0.1% using both the suspension and carrier tests is shown in Table 5. The results confirmed a significantly better activity of the two chemicals tested when used in a combination. It is evident that 0.1% concentration, which appeared insufficient in separate testing of individual chemicals, showed a reliable bactericidal activity when both chemicals were used in a combination.

The results of suspension test showed that the combination of HP and CA at lower concentrations (0.01% and 0.05%) reduced the number of all bacteria tested from 10^7 to 10^6 CFU/mL. This decrease was more noticeable for *S.* Enteritidis and *E. coli* reference strains. Significantly higher susceptibility was observed in *Staph. aureus* strain derived from sheep cheese 'Bryndza', where 0.05% concentration caused the decrease of the number of bacteria from 10^7 CFU/mL to zero. In the carrier test, the combination of HP and CA caused a moderate decrease in the number of all bacterial strains tested with the exception of *E. coli*. However, antibacterial activity was significantly improved with the use of UV light. On average, the combination of both chemicals at 0.05% concentration reduced the number of *Staph. aureus* from 10^7 to 10^6 CFU/mL (in case of *Staph. aureus* strain isolated from poultry meat even to 10^5 CFU/mL) and the number of *S.* Enteritidis reference strain from 10^6 to 10^3 CFU/mL. As to *L. monocytogenes*, the number of 10^7 CFU/mL observed in the carrier test was decreased to 10^5 CFU/mL when the tile was simultaneously exposed to UV light.

Discussion

Gram-negative bacteria are generally more resistant to biocides than Gram-positive bacteria because the cell wall structure limits the penetration of hydrophobic molecules and slows down the diffusion of lipophilic compounds. This is mainly due to lipopolysaccharides in the outer layer of Gram-negative bacteria cell walls. Diffusion and penetration of biocides through the bacterial cell wall is undoubtedly the critical point affecting their effectiveness. Despite the common mode of action, there are differences among individual species of Gram-positive and Gram-negative bacteria in terms of resistance to particular disinfectants. Extremely high level

of resistance to biocides was confirmed in *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Proteus* spp. and *Providencia stuartii*. Due to a waxy envelope, mycobacteria are generally even more resistant. Microbial spores with low metabolic and enzymatic activities possess extremely rigid outer coat consisting of lipids, dipicolinic acid and calcium; these compounds prevent penetration of disinfectants into the cell and explain their relatively extreme insusceptibility (Russel 1993, Russel 1996, McDonnel and Russell 1999).

Surface contamination in food-processing establishments varies considerably in type and composition. Therefore, it is difficult to find a chemical substance having all desired properties, such as rapid water solubility, ability to dissolve organic contaminants, emulsifying properties, and non-corrosiveness. The design of new and more efficient disinfectants based on a combination of multiple chemicals extends the spectrum of antimicrobial activity, reduces effective concentrations and eliminates the decrease in efficiency caused by surface contamination (Štefkovičová et al. 2007).

In this study, antibacterial activity of two chemicals was studied *in vitro*. The primary site of CA action at pH 7.0 is the cytoplasmic membrane of bacterial cells (Sapers et al. 2002, Huang and Chen 2011). The use of CA is safe and legally permitted in the food-processing industry (Nair et al. 2005, Kim and Rhee 2015). It is known for its antibacterial properties against a broad spectrum of Gram-positive and Gram-negative pathogens (Hulánková et al. 2013) including *Salmonella* spp. and *E. coli* O157:H7 (Chang et al. 2010).

Hydrogen peroxide is known as a strong oxidizing agent. Antimicrobial activity of HP is based on the formation of destructive free hydroxyl radicals (·OH) that can oxidize thiol groups in various enzymes and proteins (McDonnel and Russel 1999) and cause irreversible damage to essential cell components, DNA, and membrane lipids. Because of a relative safety, hydrogen peroxide is approved for extensive use in the food industry, e.g. for packaging and surface sterilization but also for milk treatment in cheese manufacturing, the processing of modified whey, or as an oxidizing agent in the production of wine, dried eggs, corn syrup and instant tea. Hydrogen peroxide at 1% concentration has already been successfully used to extend the shelf life of fresh fruits and vegetables (Sapers and Simmons (1998).

In this study, the results of suspension test with *S.* Enteritidis and *E. coli* demonstrated that low concentrations of HP are sufficed to devitalize these two Gram-negative bacteria. In the tests with *S.* Enteritidis, HP was already effective at 0.1% concentration. Antibacterial activity against the *E. coli* reference strain was observed at 0.05% concentration, lower concentrations (0.01%) were sufficient to inhibit foodborne *E. coli* strains.

As to Gram-positive bacteria, 2% HP has devitalised the reference strain of *Staph. aureus* and the two *Staph. aureus* strains isolated from poultry meat and 'Bryndza'. Hydrogen peroxide at 1 and 2% concentrations reduced the number of *Staph. aureus* by 8.1 log CFU/mL (with the exception of *Staph. aureus* strains from water and rabbit meat, in which the same effect was only observed at 3% concentration). Similar results were also obtained with *L. monocytogenes*. Some authors reported higher resistance to disinfectants of *Staph. aureus* and *L. monocytogenes* as compared to *Salmonella* spp. (Kuda et al. 2011). *Listeria monocytogenes* is a particularly resilient bacterium that is able to survive in food-processing facilities despite of stressful conditions, such as low pH, high salinity, and low temperatures. And, because of biofilm formation, it is quite difficult to eliminate this bacterium from a facility (Muhterem-Uyar et al. 2015). The permanent presence of *L. monocytogenes* on surfaces and equipment poses a more serious threat than that of sporadic contamination, because it significantly increases the likelihood of a food becoming contaminated by this pathogen. The presence of persistent *L. monocytogenes* strains has been well documented in several food-processing facilities (Leong et al. 2017).

Some studies also report high antibacterial activity of CA in a combination with other chemicals. The synergistic effect was confirmed for a combination of CA with other fatty acids (e.g. lauric acid or capric acid), or organic acids (e.g. lactic, malic or acetic acids) (Van Immerseel et al. 2006, Kim and Rhee 2013). *Escherichia coli* counts were decreased more effectively when exposed to a combination of hydrogen peroxide and lactic acid (Lin et al. 2002, Huang and Chen 2011). Combination of CA with oregano oil decreased the number of *L. monocytogenes* and addition of citric acid caused even more significant decrease (Hulánková et al. 2013). Kim and Rhee (2016) reported that medium chain fatty acids combined with edible plant essential oils are very effective against *E. coli* O157:H7. A very good antibacterial effect against *L. monocytogenes* was also reported for a combination of CA with HP (Hulánková et al. 2013, Gadotti et al. 2014). Results of this study did not confirm bactericidal effect of CA to the reference strain of *L. monocytogenes* at 0.5% concentration. However, the combination of CA with HP was already effective at concentration of 0.1%. Due to synergistic effect of HP and CA, the combination of both chemicals was effective at lower concentrations than those required for both disinfectants used separately. The combined form seems to facilitate penetration

of both components through bacterial cell membranes (Mohan et al. 2009).

The use of UV light is a possible way to increase the effect of disinfection. Antimicrobial effect of UV radiation has been confirmed for wavelengths between 100 and 300 nm, with the maximum at 260 nm. Vegetative bacterial cells are more sensitive to UV radiation than the cells of yeasts, and the spores of moulds are the most resistant (Menetrez et al. 2010) because the coats of spore-forming microorganisms contain components which protect them against UV radiation (Ondrašovič et al. 1996). The results of this study revealed an improved antibacterial activity of CA and HP when used in a combination. Synergistic effect of both chemicals has resulted in a sufficient bactericidal activity against all the reference strains as well as wild strains of selected Gram-negative and Gram-positive foodborne pathogens tested. Significant reduction of microbial counts was observed after exposure to UV radiation. To conclude, the combination of HP and CA at a concentration of 0.1% appears to be a promissable binary bactericidal formulation for potential use in the food sector.

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