



Bioactivity and morphological changes of bacterial cells after exposure to 3-(*p*-chlorophenyl)thio citronellal



Júlia Coswig Goldbeck^{a, *}, Francine Novack Victoria^b, Amanda Motta^c,
Lucielli Savegnago^d, Raquel G. Jacob^b, Gelson Perin^b, Eder João Lenardão^b,
Wladimir Padilha da Silva^a

^a Universidade Federal de Pelotas (UFPel), Faculdade de Agronomia Eliseu Maciel (FAEM), Departamento de Ciência e Tecnologia Agroindustrial (DCTA), Pelotas, RS, Brazil

^b Centro de Ciências Químicas, Farmacêuticas e de Alimentos (CCQFA), LASOL, Universidade Federal de Pelotas (UFPel), Pelotas, RS, Brazil

^c Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

^d Centro de Desenvolvimento Tecnológico, Unidade Biotecnologia, Universidade Federal de Pelotas (UFPel), Pelotas, RS, Brazil

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ABSTRACT

In order to investigate the use of semi-synthetic antimicrobial compounds, the objective of this study was to evaluate the 3-(*p*-chlorophenyl)thio citronellal, a novel compound that is derived from the essential oil of *Cymbopogon citratus*, against spoilage and pathogenic bacteria in food. This study also evaluated the possible mechanisms of action and cellular targets. Qualitative and quantitative tests and scanning electron microscopy were used to compare 3-(*p*-chlorophenyl)thio citronellal with citral and (*R*)-citronellal. It was observed that 3-(*p*-chlorophenyl)thio citronellal is a potent antimicrobial agent with bactericidal activity against different pathogenic and spoilage bacteria, of which the Gram-positive species were the most sensitive. The minimal inhibitory concentration for 3-(*p*-chlorophenyl)thio citronellal was approximately 100 times lower than that of citral, and this was corroborated by the kinetic curve of action, demonstrating that cell death was faster when the bacterial cells were treated with 3-(*p*-chlorophenyl)thio citronellal. This finding showed that the addition of thiol enhances the bioactivity of citral. In vitro testing showed that the compound does not show toxicity. The SEM images showed cell damage with the formation of pores in the cell wall and membrane, which are possibly the cellular targets of the compound.

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1. Introduction

Due to the constant search for safe food practices, many studies have sought to find new compounds with antimicrobial activities because of reports that the frequent use of synthetic preservatives may be toxic and carcinogenic (Guillard, Issoufov, Redl, & Gontard, 2009; Mpountoukas, Vantarakis, Sivridis, & Lialiaris, 2008).

These data, among many others, confirm the urgent need to discover new antimicrobial compounds for use in foods, which is a fact that is already recognized by the World Health Organization (Bassolé & Juliani, 2012; Carson, Hammer, & Riley, 1995; Frymoyer, Hersh, Coralic, Benet, & Joseph Guglielmo, 2010; Gill, Delaquais, Russo, & Holley, 2002).

In this context, the utilization of essential oils as antimicrobial substances has provided an alternative to synthetic preservatives as an ecofriendly way to increase the shelf life and safety of foods (Mpountoukas et al., 2008). However, although their antimicrobial activity has been experimentally demonstrated by researchers, essential oils are highly volatile substances, which limit their direct use as food preservatives (Braga, Silveira, Zeni, Severo, & Stefani, 1996; Hawser et al., 2011; Leonard, Virijevic, Regnier, & Combrinck, 2010; Tyagi & Malik, 2010).

In recent decades, research on the possible applications of organochalcogen compounds in the area of food science and technology has become prominent because these compounds are very attractive due to their large spectrum of biological activity, which includes their antioxidant, anticancer, antimicrobial, antiviral, antidepressant-like, anti-ulcer, neuroprotector, antinociceptive and antiinflammatory properties (Gutiérrez, Battle, Sánchez, & Nerín, 2010; Nogueira, Zeni, & Rocha, 2004;

* Corresponding author. Tel./fax: +55 53 3275 7258.

E-mail address: juliaqa86@yahoo.com.br (J.C. Goldbeck).

Savegnago et al., 2006; Wilhelm, Jesse, Bortolatto, Nogueira, & Savegnago, 2009).

Our research group has been working on the chemical modification of the major compounds of essential oils and functionalizing these bioactive molecules with organochalcogens such as selenium and sulfur. Many studies have focused on the antimicrobial activity of the essential oil compounds against pathogens and spoilage bacteria commonly found in food. Studies have also attempted to reduce the volatility and the sharp smell of essential oils because it is well known that these characteristics are disadvantages of the application of essential oils in food products. Our research group recently designed a protocol for the green synthesis of new organochalcogen compounds derived from citral, the major compound present in the essential oil of *Cymbopogon citratus*. Furthermore, this protocol is considered a green protocol because it minimizes the use of solvent, the products are derived of a renewable source, and it uses microwave irradiation to accelerate the reaction. The preliminary studies of those compounds showed good antibacterial activity against *Staphylococcus aureus* strains (Lenardão, Ferreira, Jacob, Perin, & Leite, 2007).

The goal of this study was to qualitatively and quantitatively evaluate the bioactivity and kinetics of action of 3-(*p*-chlorophenyl)thio citronellal against bacteria of importance in regard to foods, using citral and (*R*)-citronellal (a structural analogue of the modified compound) as controls. Moreover, it was studied the morphological changes of bacterial cells in contact with this new antimicrobial agent, to determine a possible mechanism of action for this compound.

2. Material and methods

2.1. Synthesis of 3-(*p*-chlorophenyl)thio citronellal

The synthesis of 3-(*p*-chlorophenyl)thio citronellal was performed according to the methodology developed by our research group (Lenardão et al., 2007). At room temperature, KF/Al₂O₃ (50%) was added to a mixture of citral (1 mmol) and *p*-chlorothiophenol (1.2 mmol). The whole mixture was stirred for 1 min and then irradiated with a microwave for 6 min. The product was purified by column chromatography over silica gel using hexanes/acetate (97:3) as eluent.

2.2. In vitro toxicity

The toxicity of 3-(*p*-chlorophenyl)thio citronellal *in vitro* was determined via the activity of the enzyme δ -Ala-D in mouse livers. The assay was performed according to the methods proposed by Sassa (1982), which measured the rate of product formation (porphobilinogen) using 84 mM phosphate buffered saline (PBS OXOID[®]) (pH 6.4) and 2.5 mM aminolevulinic acid (Sigma).

This reaction is related to situations associated with oxidative stress. All of the assays were performed after 10 min of pre-incubation. The samples were incubated for 30 min at 37 °C, and

the reaction product was determined at 555 nm using the modified Ehrlich reagent with a molar absorption coefficient of $6.1 \times 10^4 \text{ cm}^{-1} \text{ M}^{-1}$ to porphobilinogen Ehrlich salt.

2.3. Antimicrobial activity against bacteria of importance in foods

Tests were carried out qualitatively and quantitatively against Gram-positive and Gram-negative pathogenic and spoilage bacteria commonly found in foods to evaluate the spectrum of action of 3-(*p*-chlorophenyl)thio citronellal because most synthetic preservatives have low spectra of action.

In this study citral, (*R*)-citronellal [structure analogous to 3-(*p*-chlorophenyl)thio citronellal] and 3-(*p*-chlorophenyl)thio citronellal (Fig. 1) were employed to observe whether the addition of thiol enhances the antimicrobial activity of citral, which was determined by comparing the bioactivity between the compounds.

2.3.1. Agar diffusion method

The agar diffusion method of the *Clinical and Laboratory Standards Institute – NCCLS* (2012a, 2012b) was employed with some modifications as a qualitative test to evaluate the antimicrobial activity of 3-(*p*-chlorophenyl)thio citronellal, citral and (*R*)-citronellal. The modifications included using prepared discs of filter paper with a 6 mm diameter to which 5 μL of the compound to be tested was added, starting with an initial concentration of 2 mg mL⁻¹. The antimicrobial activity was tested in triplicate against the pathogenic bacteria *Listeria monocytogenes* (ATCC 7644), *Shigella dysenteriae* (ATCC 4002), *Escherichia coli* (ATCC 11775), *Salmonella* Typhimurium (ATCC 1408) and *Staphylococcus aureus* (FRI 326) and against the spoilage bacterium *Pseudomonas fluorescens* (INCQS 00077), which were provided from a bank of bacterial strains by the Foundation Oswaldo Cruz, Brazil. This stage was performed in the Food Microbiology Laboratory in the Department of Science and Technology of Foods at the Federal University of Pelotas/RS, Brazil.

The cellular concentrations of bacterial cultures were standardized to a cell density of $1.5 \times 10^8 \text{ CFU mL}^{-1}$ (0.5 McFarland).

2.3.2. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the broth microdilution method according to the protocol recommended by the NCCLS (2012b) with some modifications. Microdilutions were performed in triplicate. The tests were performed on the same bacterial strains as those used in 2.3.1 Initial concentrations of 13 mM citral, 13 mM (*R*)-citronellal and 7 mM 3-(*p*-chlorophenyl)thio citronellal were added to tryptic soy broth that was supplemented with 0.6% yeast extract (TSB-YE-OXOID[®]). The concentrations of bacteria were adjusted to $1.5 \times 10^8 \text{ CFU mL}^{-1}$ (0.5 McFarland).

In a microdilution plate, were added 100 μL of the inoculum and 100 μL of the compound to be tested and incubated at 37 °C for 24 h. After incubation, 10 μL was added to 3% resazurin (Sigma) in each dilution, and the broth microdilution plate was incubated at 37 °C

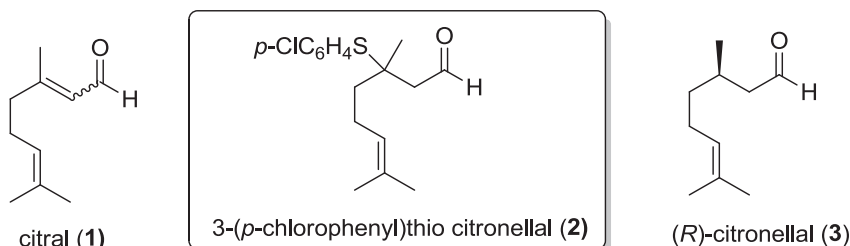


Fig. 1. Chemical structure of the compounds evaluated in this study.

for 30 min. Resazurin is a redox indicator and has been used to assess viability and bacterial contamination (Smith & Townsend, 1999). A sample containing only the inoculum represented the positive control, and a sample containing only TSB-YE represented the negative control. The MIC of an antimicrobial agent is defined as the lowest concentration of a compound that results in no visible growth after 24 h of incubation.

In another microplate containing 100 μL in each cavity was added 100 μL from each well where no cell growth in the microplate of MIC and incubated again for 24 h at 37 °C. Those wells where no cell multiplication indicates bactericidal activity.

2.4. Behavior of Gram-positive and Gram-negative bacteria following treatment with 3-(*p*-chlorophenyl)thio citronellal

The Gram-positive pathogenic bacteria *L. monocytogenes* (ATCC 7644) and the Gram-negative bacteria associated with spoilage in foods *P. fluorescens* (INCQS 00077) were selected to evaluate cell morphology following exposure to 3-(*p*-chlorophenyl)thio citronellal, citral and (*R*)-citronellal via scanning electron microscopy.

2.4.1. Kinetics of antimicrobial action

To evaluate the kinetics of action of the compounds against Gram-positive and Gram-negative bacteria, 2 mL of inoculum at a concentration of 1.5×10^8 CFU mL⁻¹ was added to a test tube containing 100 μL of the antimicrobial solution at a concentration of 0.21 mM 3-(*p*-chlorophenyl)thio citronellal (against *L. monocytogenes*), 0.42 mM 3-(*p*-chlorophenyl)thio citronellal (against *P. fluorescens*) and 1.6 mM citral and (*R*)-citronellal (against both bacteria). The concentrations were predefined based on the MIC values. Count (CFU) was determined after 0, 5, 10, 15, 20, 30, 40, 50, 60, 80, 90, 100 and 120 min of incubation at 37 °C. At each incubation time, decimal dilutions were performed to 10⁻⁶ CFU mL⁻¹. The calculation of CFU was performed by adding 20 μL of each dilution to plates that contained trypticase soy agar (TSA-OXOID®), which were then incubated at 37 °C for 24 h. After this period, the colonies were counted and the kinetic action curve was constructed (Motta & Brandelli, 2002).

2.4.2. Analysis of cellular morphology using scanning electron microscopy (SEM)

To evaluate the possible mechanisms of action and cellular targets of the compounds against the Gram-positive and Gram-negative bacteria selected, a morphological analysis of the bacteria was carried out following treatment with the antimicrobial compounds. An aliquot of 2 mL of the standardized inoculum was adjusted to a 0.5 McFarland scale, which corresponded to 1.5×10^8 CFU mL⁻¹. The inoculum was then added to 0.21 mM 3-(*p*-chlorophenyl)thio citronellal (against *L. monocytogenes*), 0.42 mM 3-(*p*-chlorophenyl)thio citronellal (against *P. fluorescens*) and 1.6 mM citral and (*R*)-citronellal [against both bacteria (MIC of each compound)]. Thereafter, it was incubated at 37 °C for 1 h, which were the temperature and time required for cell death to occur based on the data provided by the construction of the kinetic curve.

The suspension was centrifuged at 3000 g for 15 min. For the scanning electron microscopy analysis, cells were fixed with 2% glutaraldehyde in NA-cacodylate buffer (100 mM, pH 7.1). The cells were pelleted, washed to remove the glutaraldehyde and suspended in the same buffer. A drop of each suspension was transferred to poly-L-lysine to make the chips, which were kept in a hydration chamber for 30 min for cell adhesion. The attached cells were fixed by immersion in 1% osmium tetroxide (OsO₄) in cacodylate buffer for 30 min, washed with the same buffer and dehydrated in increasing concentrations of ethanol, 50%, 70%, 95% (2 \times) and 100% (2 \times), for 10 min each. The chips were mounted on aluminum stubs and coated with gold in a sputter coater (Emitech K5550, Ashford, Kent, UK). The chips were viewed at 15 kV accelerating voltage in a scanning electron microscope (JEOL JSM - 6060®) according to the method described by Kalchayanand, Dunne, and Ray (2004).

2.5. Statistical analysis

The experimental results were given as the mean \pm standard deviation (SD) and were analyzed by ANOVA followed by Newman–Keuls test for multiple comparisons when appropriate. The differences between groups were considered significant when $p < 0.05$. All tests were performed in triplicate.

3. Results and discussion

3.1. In vitro toxicity

Based on the antimicrobial activity displayed by 3-(*p*-chlorophenyl)thio citronellal, its hepatotoxic effects were investigated, searching for future technological applications in food preservation.

It is noted that different concentrations (10, 50, 100 and 500 μM) of 3-(*p*-chlorophenyl)thio citronellal do not inhibit the enzyme activity of δ -Ala-D, which suggests the absence of the compound's toxicity *in vitro* in mouse livers (data not shown).

3.2. Antimicrobial activity of 3-(*p*-chlorophenyl)thio citronellal

The *in vitro* bioactivity of citral, (*R*)-citronellal and 3-(*p*-chlorophenyl)thio citronellal against bacteria of importance in food was qualitatively assessed via the presence or absence of zones of inhibition in agar (Table 1) and quantitatively assessed via the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), as described in Table 2.

All microorganisms tested were shown to be potentially sensitive to compounds with mean inhibition zones of between 14.7 and 39.3 mm in diameter.

Based on the data shown in Table 1, 3-(*p*-chlorophenyl)thio citronellal has a broad action spectrum against Gram-positive and Gram-negative pathogens. These results are consistent with other studies conducted by our research group, which proves that the compounds obtained from the chemical modification of essential

Table 1
Antimicrobial activity of compounds citral A, (*R*)-citronellal B and 3-(*p*-chlorophenyl)thio citronellal B by the agar diffusion method.

	Inhibition zone (mm)					
	<i>S. Typhimurium</i>	<i>S. aureus</i>	<i>S. dysenteriae</i>	<i>L. monocytogenes</i>	<i>E. coli</i>	<i>P. fluorescens</i>
A	34.7 \pm 1.2	34.7 \pm 0.6	35.3 \pm 0.3	34.3 \pm 0.9	33.7 \pm 1.3	25.3 \pm 0.7
B	19.0 \pm 0.6	18.7 \pm 0.7	20.3 \pm 1.5	20.3 \pm 0.9	24.7 \pm 0.7	14.7 \pm 1.2
C	35.7 \pm 0.6	40 \pm 0.0	39 \pm 0.6	39.0 \pm 0.5	39.3 \pm 0.7	31.0 \pm 1.0

A: Citral B: (*R*)-citronellal C: 3-(*p*-chlorophenyl)thio citronellal.
Each value is the mean \pm SEM ($n = 3$).

Table 2
Minimum inhibitory concentration (mM) and minimum bactericidal concentration (mM) of citral A, (R)-citronellal B and 3-(p-chlorophenyl)thio citronellal C against standard screening strains of bacteria.

	<i>S. Typhimurium</i>		<i>S. aureus</i>		<i>S. dysenteriae</i>		<i>L. monocytogenes</i>		<i>E. coli</i>		<i>P. fluorescens</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
A	16	4.2	16	4.2	16	16	16	3.3	16	16	16	3.3
B	16	4.2	16	4.2	16	16	16	3.3	16	16	16	3.3
C	4.2	2.1	2.1	2.1	4.2	4.2	2.1	2.1	4.2	4.2	4.2	4.2

A: Citral B: (R)-citronellal C: 3-(p-chlorophenyl)thio citronellal.

oils derived from plants have the potential to be used as preservatives in food products. These compounds can be an environmentally friendly alternative to synthetic preservatives, which in most cases, are effective against only a few bacterial species and therefore have a low spectrum of action (Lenardão et al., 2009).

The MIC values for the 3-(p-chlorophenyl)thio citronellal inhibitory activity against the bacteria used in this study showed that the Gram-positive bacteria (*Listeria monocytogenes* and *Staphylococcus aureus*) are more sensitive than the Gram-negative bacteria (*S. Typhimurium*, *S. dysenteriae*, *Escherichia coli* and *Pseudomonas fluorescens*), with average MIC values of 2.1 mM for Gram-positive and 4.2 mM for Gram-negative bacteria (Table 2).

The results are in agreement with other studies that have shown that Gram-positive bacteria are more sensitive than Gram-negative bacteria to essential oils (Cosentino et al. 1999; Dorman & Deans, 2000; Farag, Daw, Hewedi, & El-Baroty, 1989; Juliano & Mattana et al. 2000; Lambert et al. 2001; Thoroski, Blank, & Biliaderis, 1989).

Moreover significant differences between the MIC values of 3-(p-chlorophenyl)thio citronellal and the values observed for citral and (R)-citronellal showed that the addition of thiol potentiates the bioactivity of citral. However, the MIC values for 3-(p-chlorophenyl)thio citronellal in the Gram-positive *Listeria monocytogenes* and the Gram-negative *Pseudomonas fluorescens* were almost 100 times larger than those for citral and (R)-citronellal.

Aldehydes, such as citral, exhibit antimicrobial activity due to the inactivation of proteins through the formation of covalent bonds with organic groups (Tortora, 2005). It is assumed that the increase in bioactivity of citral after the addition of sulfur in the molecule may be explained by the interaction with the sulfur amino acids that comprise the bacterial cell wall or membrane, which would result in the formation of disulfide bonds. Thus, it is possible that 3-(p-chlorophenyl)thio citronellal may destabilize the membrane or bacterial cell wall more easily than citral.

The MBC results for the compounds against all of the bacterial strains tested showed that these substances possess a potent bactericidal effect.

3.3. Analysis of the behavior of *L. monocytogenes* and *P. fluorescens*

The qualitative and quantitative tests indicated that Gram-positive and Gram-negative bacteria exhibit different behaviors towards the compounds tested. Thus, the kinetics of action was evaluated and the cell morphology was analyzed via scanning electron microscopy (SEM), using the Gram-positive pathogen *L. monocytogenes* and the deteriorating Gram-negative bacterium *P. fluorescens* as targets.

Fig. 2 and Fig. 3 graphically shows the effect on the two tested bacterial species over time after exposure to three different antimicrobials, evaluating their multiplication rate expressed in CFU mL⁻¹.

Figs. 2 and 3 demonstrates that the number of viable cells decreased in proportion to the exposure time of *Listeria monocytogenes* and *Pseudomonas fluorescens* to 3-(p-chlorophenyl)thio citronellal. In the first 5 min of contact between the compound and the bacterial cells, there was a significant reduction in the number of cells, which reduced the logarithmic cycle and highlights the potent bactericidal action of this compound. Interestingly, the times required for the total kill of the bacterial cells were significantly different among the three compounds evaluated and were lowest for 3-(p-chlorophenyl)thio citronellal.

When evaluating the kinetic curve of antimicrobial compounds against *L. monocytogenes* (Fig. 2), it was observed that cell death occurred in only 15 min after contact with 3-(p-chlorophenyl)thio citronellal. When the cells were treated with citral and (R)-citronellal, 40 and 50 min, respectively, were necessary for the same antibacterial effect. Regarding the kinetics of action of compounds

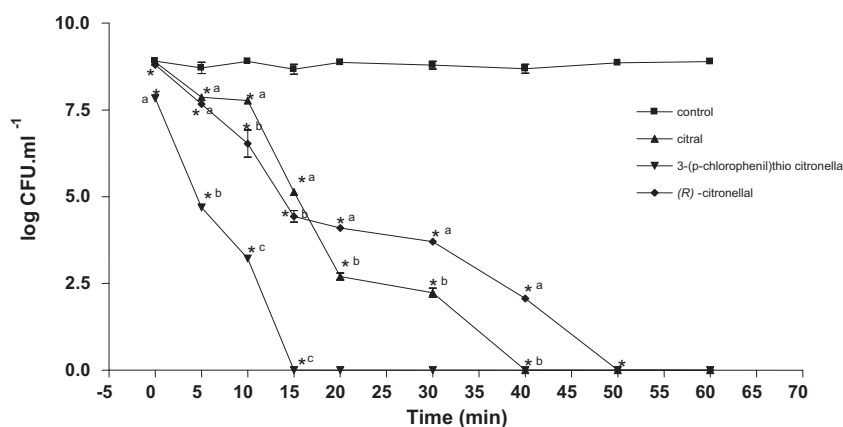


Fig. 2. Inactivation kinetics of compounds on *Listeria monocytogenes*. Each value is expressed as mean \pm SEM ($n = 3$). Asterisks represent significant effect ($*p < 0.05$) as compared with the respective control (C) without compound. Small letters (a) represent significant effect ($p < 0.05$) as compared with p-chlorophenylthio citronellal and (b) represent significant effect (R)-citronellal ($p < 0.05$) as compared with by Newman–Keuls multiple range test's.

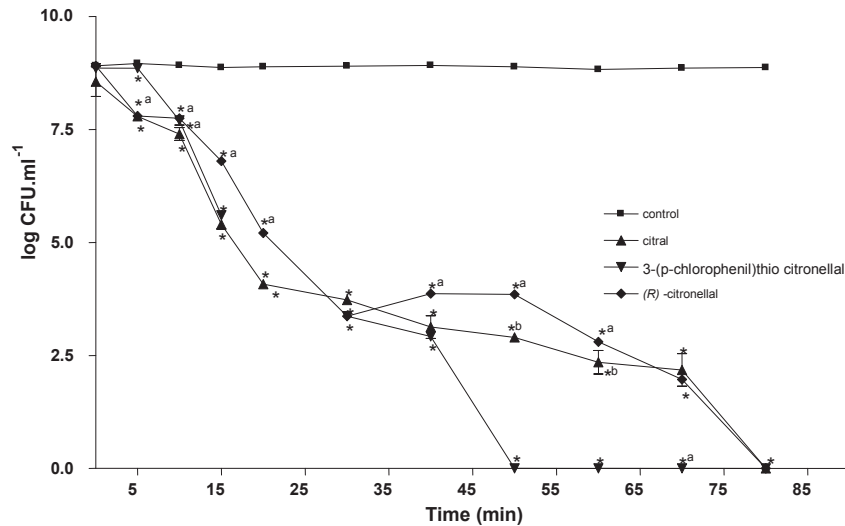


Fig. 3. Inactivation kinetics of compounds on *P. fluorescens*. Each value is expressed as mean \pm SEM ($n = 3$). Asterisks represent significant effect ($*p < 0.05$) as compared with the respective control (C) without compound. Small letters represent significant effect ($p < 0.05$) as compared with 3-(*p*-chlorophenyl)thio citronellal and represent significant as compared with by Newman–Keuls multiple range tests.

against *P. fluorescens* (Fig. 3), the cell death occurred in 33 min following contact with 3-(*p*-chlorophenyl)thio citronellal, whereas it took 75 min of contact with citral and (*R*)-citronellal for cell death to occur.

Gram-negative bacteria were more resistant to the test compounds compared with Gram-positive bacteria, which confirms the results obtained in qualitative and quantitative tests where the Gram-positive bacteria were more sensitive. Indeed, Bassolé et al. (2011) and Naik, Fomda, Jaykumar, and Bhat (2010) studied the antimicrobial activity of the essential oil of *Cymbopogon citratus* against different bacteria with importance in foods and showed that Gram-negative *Pseudomonas aeruginosa* are potentially resistant to oil.

These results demonstrate that 3-(*p*-chlorophenyl)thio citronellal has strong antibacterial activity that quickly acts on Gram-positive and Gram-negative bacteria, even at low concentrations.

The low effective concentrations, broad spectrum of action and quick action of 3-(*p*-chlorophenyl)thio citronellal observed in the present study are relevant to its application in commercial foods.

3.4. Analysis of cellular morphology using scanning electron microscopy (SEM)

The potent bactericidal activity of 3-(*p*-chlorophenyl)thio citronellal against *L. monocytogenes* and *P. fluorescens* was compared via an analysis of the cell morphology of these microorganisms using scanning electron microscopy (SEM) following treatment with this compound. Control cells were not treated with these compounds (Fig. 4).

The morphological change was apparent in the cells of both pathogens that were exposed to the antimicrobial compound, in

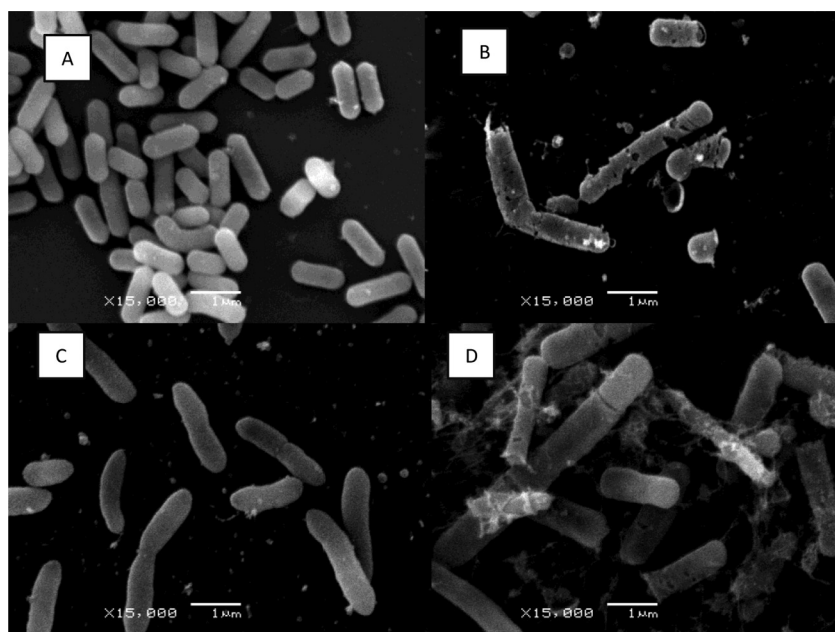


Fig. 4. Morphological analysis by scanning electron microscopy. (A) Standard cell morphology of *Listeria monocytogenes*. (B) Cells of *L. monocytogenes* treated with 3-(*p*-chlorophenyl)thio citronellal. (C) Standard cell morphology of *Pseudomonas fluorescens*. (D) Cells of *P. fluorescens* treated with 3-(*p*-chlorophenyl)thio citronellal.

which destruction of the cells and formations of pores in the cell wall were observed (Fig. 4).

Some researchers report that the possible mechanisms of action of essential oils and their derivatives include the degradation of the cell wall, cytoplasmic membrane damage, damage to membrane proteins, leakage of intracellular content, coagulation of the cytoplasm and/or depletion of proton motive force (Bassolé & Juliani, 2012; Skandamis, Koutsoumanis, Fasseas, & Nychas, 2001).

Based on an analysis of morphological images, it is inferred that the cell wall and membrane are the main targets of 3-(*p*-chlorophenyl)thio citronellal. It is believed that the mechanism of action of the compound may involve preventing the connection of N-acetyl muramic acid (NAM), which would block the synthesis of the cell wall.

Based on this possible mechanism of action, the greater resistance of Gram-negative bacteria may be associated with the presence of the outer membrane, which hinders access to the bacterial inner membrane, a probable cellular target of the compound. Given the affinity of essential oils and their derivatives towards the inner membrane lipids of bacteria, these compounds can easily reach the internal milieu of the cell. Because Gram-positive bacteria do not possess the outer membrane, they become more sensitive to these compounds (Bassolé & Juliani, 2012; Cox et al., 1998; Gustafson et al., 1998; Helander et al., 1998; Lambert et al., 2001; Oosterhaven, Poolman, & Smid, 2005; Skandamis et al., 2001; Smith-Palmer, Stewart, & Fyfe, 1998).

Rasooli et al. (2006) analyzed morphological changes in the cells of *Listeria monocytogenes* treated with essential oil of thyme (*Thymus vulgaris*) via transmission electron microscopy and observed that the cells had reduced size and were more clustered, forming small buds on the cell wall, which is similar to the observations in the present study.

4. Conclusions

It can be concluded that 3-(*p*-chlorophenyl)thio citronellal, citral and (*R*)-citronellal, even at low concentrations, exert bactericidal effects and cause different morphological changes in Gram-positive and Gram-negative bacteria. Therefore, these compounds may be potential antimicrobial agents against some bacteria with importance in foods.

The addition of thiol enhances the bioactivity of citral, which functionalizes the molecule. Thus, 3-(*p*-chlorophenyl)thio citronellal is a potent bactericidal compound with low toxicity, and may, therefore, have the potential to be used in foods.

These results contribute significantly to advances in the knowledge of the bioactivity of essential oils and their chemical modifications for use as antibacterial agents in foods. This study aids in understanding the mechanism of action of these new agents, because previous research studies have only proposed suggestions concerning their modes of action. The cellular wall and membrane are possibly the main targets of 3-(*p*-chlorophenyl)thio citronellal.

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