



Antioxidant properties of coriander essential oil and linalool and their potential to control *Campylobacter* spp.



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ABSTRACT

Foodborne diseases remain common around the world with *Campylobacteriosis* being the most commonly reported zoonosis in the European Union in 2013. *Campylobacter jejuni* and *Campylobacter coli* are the main species associated with human illness. Furthermore, *Campylobacter* can develop biofilms which is becoming a major problem within the food industry. In addition to foodborne pathogens, oxidation is a non-microbial cause of deterioration of food causing loss of quality and safety. Thus, there is an urgent need in the food industry for new and effective strategies that can help prevent food contamination, spoilage and consequently, foodborne illnesses. Essential oils are known for their antimicrobial and antioxidant properties and are already widely used in the food industry. So, the aim of this work was to study the antimicrobial and anti-biofilm activity of coriander essential oil and its major compound linalool against *C. jejuni* and *C. coli* strains, as well as their effect in the quorum sensing (QS) system and their potential as antioxidants. Our results, demonstrated that both compounds have anti-*Campylobacter* activity, inhibited *in vitro* biofilm formation and promoted biofilm dispersion even at sub-MIC concentrations and interfered with the QS system through the inhibition of violacein production. Moreover, the essential oil and linalool were shown to have radical scavenging properties and lipid peroxidation inhibition ability which could make them potential alternatives to synthetic antioxidants. In sum, our results demonstrated the antibacterial, anti-biofilm, anti-QS and antioxidant potentials of the coriander essential oil and its major compound, linalool, suggesting that they could be used in the food industry to enhance shelf life of food products and increase food safety without requiring chemical additives or preservatives.

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1. Introduction

Despite advances in food safety, foodborne diseases remain common throughout the world and a growing threat to global public health. The ingestion and/or contact with contaminated food are the major cause of foodborne illness (CDC, 2015; EFSA & ECDC, 2015). *Salmonella*, *Escherichia coli* and *Campylobacter* are the three major pathogens that cause foodborne illnesses in United States (CDC, 2015), while *Campylobacteriosis* was the most commonly reported zoonosis in the European Union in 2013 (EFSA & ECDC, 2015). *Campylobacteriosis* is usually associated to the consumption of undercooked meat, environmental contamination, and

cross-contamination between raw and cooked food (Hardy, Lackey, Cannon, Price, & Silbergeld, 2011; Silva, Leite, Fernandes, Mena, Gibbs, & Teixeira, 2011). Poultry is considered to be the main source of food-related human campylobacteriosis (Corry & Atabay, 2001; EFSA & ECDC, 2015). The most common species of *Campylobacter* associated with human illness are *Campylobacter jejuni* and *Campylobacter coli* (Fitzgerald, 2015). In addition, it has also been described that *Campylobacter* could develop biofilms that are more resistant to disinfectants and thus becoming a major problem within the food industry (Gunther & Chen, 2009; Srey, Jahid, & Ha, 2013). Quorum sensing (QS) communication is associated to the bacterial biofilm formation and antibiotic resistance, as well as to the bacterial proliferation in foods and food spoilage, therefore, QS inhibition could be a good strategy to control *Campylobacter* and to ensure food safety (Alvarez, Moreira, & Ponce, 2012; Götz, Sharbati, Backert, & Alter, 2012; Nazzaro, Fratianni, &

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Coppola, 2013). It is thus extremely important to search for new and effective measures to reduce *Campylobacter* proliferation in food and thus reduce the incidence of *Campylobacter* infection in humans.

In addition to the foodborne pathogens, oxidation is a well-known non-microbial cause of deterioration of food and loss of quality and safety (Falowo, Fayemi, & Muchenje, 2014; Sanches-Silva et al., 2014). Oxidative stress occurs due to the irregular generation of free radicals reactive oxygen species which triggers the oxidative stress and damage to macromolecules such as the lipid and protein fractions of food products (Falowo et al., 2014). Since antioxidants are compounds that interact with free radicals and prevent the oxidation of other molecules, finding compounds that have both antimicrobial and antioxidant activities has a great potential for the application in food systems (Sanches-Silva et al., 2014). There has been a growing interest in the use of natural compounds for application in food products, mainly due to the preference of consumers for natural ingredients and the concerns about the toxic effects of synthetic compounds (Falowo et al., 2014).

Essential oils are known for their antimicrobial and antioxidant properties and their use in the food industry has been widely described (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Calo, Crandall, O'Bryan, & Rieke, 2015; Falowo et al., 2014; Jayasena & Jo, 2013; Sanches-Silva et al., 2014; Silva & Domingues, 2015). In short, essential oils are very complex natural mixtures and are characterized by two or three major components at fairly high concentrations (20–70%), such as linalool ($\approx 68\%$) that is the major component of the coriander essential oil (Bakkali et al., 2008; Silva, Ferreira, Duarte, Mendonça, & Domingues, 2011). The antioxidant effects of coriander oil have been described, suggesting that this oil could be considered as a source of natural antioxidants and used as a potential substitute for synthetic antioxidants in the food industry (Laribi, Kouki, M'Hamdi, & Bettaieb, 2015; Samojlik, Lakić, Mimica-Dukić, Daković-Švajcer, & Božin, 2010; Silva & Domingues, 2015; Singh, Kapoor, Pandey, Singh, & Singh, 2002). The bactericidal effect and the anti-biofilm potential of coriander essential oil against several pathogens has also been described (Duarte, Ferreira, Oliveira, & Domingues, 2013; Lo Cantore, Iacobellis, De Marco, Capasso, & Senatore, 2004; Silva & Domingues, 2015; Silva, Ferreira, Queiroz, & Domingues, 2011). In addition, the potential of coriander oil to control *C. jejuni* in raw meat has already been described (Rattanachaiakunsopon & Phumkhachorn, 2010). Nevertheless, the effect against *C. coli* has not yet been described, as well as the potential of this essential oil to prevent or eliminate biofilms formed by these foodborne pathogens.

So, the aim of this work was to study the antimicrobial and anti-biofilm activity of coriander essential oil and its major compound linalool against *C. jejuni* and *C. coli* strains. Finally, the role of coriander oil and linalool in the QS system was also studied, as well as its potential as an antioxidant.

2. Materials and methods

2.1. Antibacterial agents and bacterial strains

Coriander essential oil and its major compound linalool (obtained from Sigma–Aldrich St. Louis, MO) were tested against two reference strains (*C. jejuni* ATCC 33560 and *C. coli* ATCC 33559) and two *Campylobacter* isolates (*C. jejuni* 225421 and *C. coli* 873) (Duarte et al., 2014). The strains were stored in Brain Heart Infusion broth with 20% glycerol at -80°C and prior to susceptibility testing each strain was inoculated on Brucella agar plates (Oxoid, England) supplemented with 5% defibrinated horse blood to ensure optimal growth. The chemical composition of commercial coriander

essential oil is reported in the literature, being linalool (64.38%), geranyl acetate (5.82%), camphor (4.88%), p-cymene (4.54%) and α -pinene (4.04%) the major compounds identified (Silva, Ferreira, & Duarte et al., 2011).

2.2. Antibacterial activity

2.2.1. Disc diffusion test

The susceptibility of the *Campylobacter* strains to the antimicrobials was evaluated by the disc diffusion test, according to the standard M2-A8 from Clinical Laboratory Standards Institute (CLSI, 2003). The pure compounds (10 $\mu\text{L}/\text{disc}$) and a 50% (v/v) solution of the compound in dimethyl sulfoxide (DMSO) (10 $\mu\text{L}/\text{disc}$) were added to the discs (6 mm of diameter). DMSO was used as control. The inoculum was prepared in a sterile solution of 0.85% sodium chloride and the optical density of the suspension was adjusted to 0.5 McFarland ($\approx 1 \times 10^8$ colony forming units (CFU)/mL). Then the Müller-Hinton Agar (MHA, LiofilChem, Italy) plates were inoculated, allowed to dry and the discs previously prepared were placed over the agar. The plates were incubated at 37°C for 48 h under microaerobic conditions and after incubation the diameter of the inhibition zone was measured. This assay was performed in triplicate.

2.2.2. Vapor-phase antimicrobial activity

The effect of the vapor of coriander essential oil and linalool against *Campylobacter* was evaluated as described by Fisher and Phillips (2006). Briefly, MHA plates inoculated as previously described were exposed to vapors by placing one disc impregnated with 10 μL of the antibacterial compounds onto the lid of the Petri dish (approximately 8 mm from the bacteria). Then, the plates were incubated at 37°C for 48 h under microaerobic conditions and the zones of inhibition were measured (diameter in mm). This assay was performed in triplicate.

2.2.3. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for coriander essential oil and linalool were determined by the microdilution method as described by Duarte, Martinho, Luís, Figueiras, Oleastro, and Domingues (2015). Briefly, serial two-fold dilutions of coriander oil and linalool (from 32 to 0.25 $\mu\text{L}/\text{mL}$) were prepared in 96-well plates in Müller Hinton broth (MHB) and a maximum of 2% of DMSO was used to increase the solubility. Bacterial suspensions were prepared with a turbidity of 0.5 McFarland, diluted in MHB and added to each well to yield a final concentration of 5×10^5 CFU/mL per well. DMSO and culture medium were used as growth controls. The plates were incubated at 37°C for 48 h under microaerobic conditions and after the incubation the growth was visually assessed. The MIC was defined as the lowest concentration of compound without visible growth. From the wells without visible growth, 10 μL were plated and after incubation the number of colonies was counted. The MBC was defined as the lowest compound concentration which caused the death of 99.9% of the bacterial inoculum. At least three independent assays were performed.

2.3. Anti-biofilm activity

The effect of coriander essential oil and linalool on the ability of *Campylobacter* to form biofilms was determined following the protocol described by Duarte, Alves, Ferreira, Silva, and Domingues (2015). Briefly, the bacterial suspensions were prepared from overnight cultures in MHB at 37°C under microaerobic conditions

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